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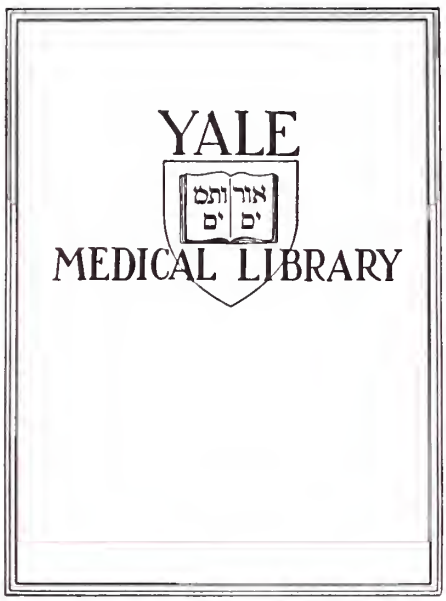
MORPHOLOGICAL AND PHYSIOLOGICAL ADAPTATION IN RAT COLONIC MUCOSA

Jerald (Mac) D. Hansing

1978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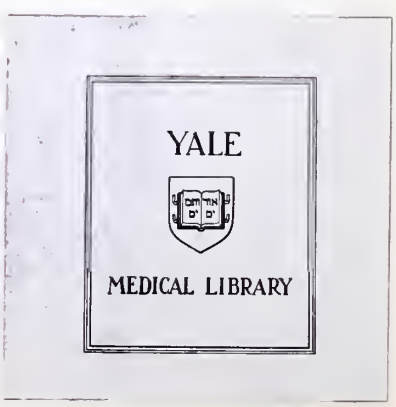
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Morphological and Physiological Adaptation in Rat Colonic Mucosa

by Jerald (Mac) D. Hansing, 1978

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the degree of Doctor of Medicine.

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Jerald (Mac) D. Hansing

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INTRODUCTION

A. Early History

The study of intestinal adaptation following surgical resections has been pursued for nearly a century. Though no one disputes the actual manifestation of adaptation, considerable controversy has persisted in attempting to describe the controlling mechanisms by which adaptation occurs.

Senn (1) was the first to describe compensatory hypertrophy in the remaining intestine following enterectomy in the dog in 1886. Flint (2) was among the first to document a review of intestinal adaptation following 59 procedures of small bowel resections from 1881 to 1912. He demonstrated that the stimulated growth in the intestine following resection was not manifested by an increase in longitudinal length, but rather by an increase in intestinal diameter. He did not find an increase in the number of villi, but did detect a decrease in the number of villi per unit area, concluding that compensatory hypertrophy, in terms of either cellular hypertrophy or cellular hyperplasia, was manifested by an increase of villus size. After performing 30-80% resections in dogs, he was able to demonstrate that the resulting villus hypertrophy of the remaining small bowel accounted for an increase in the surface area equal to the amount lost in the resection.

In 1914, Stassoff (3) was able to demonstrate that small bowel resection also resulted in functional adaptation: by collecting chyme from a jejunal fistula following distal resection in the dog, he found a progressive decrease with time in the quantities of fat, starch, and soluble nitrogen recovered from the chyme.

B. Brief Review of Controversy over Control Mechanisms of Intestinal Adaptation

Interest in intestinal adaptation has been renewed in the past decade, particularly over the controversy of attempts to describe the control mechanisms behind adaptation. Essentially four schools of thought have emerged, which will be summarized here but elaborated upon in more detail in the discussion of this report.

The first school of thought has emphasized the influencial factors of intraluminal contents as initiating adaptation responses. Three factors of intraluminal contents have been implicated:

1. Booth et. al. (4) formulated the "dietary bulk hypothesis" in 1959 as the stimulus for adaptation.
2. Dowling and Booth (5) then modified an adaptation theory based upon the stimulus of intraluminal nutrition in 1967.
3. Finally, Altmann and LeBlond (6) in 1970 considered the possible role of endogeneous hormones secreted intraluminally from pyloric, biliary, pancreatic, or duodenal sources as the stimulus for adaptation.

The second school of thought focused upon neurovasculature influences of adaptation. Ballinger (7) watched for the development of adaptation responses following denervation, and Touloukian (8) studied the effects of local perfusion upon adaptation.

The "tissue mass hypothesis" was developed as another school of thought based upon the works of Weiss (9) in 1957. Weiss had developed mathematical models which were manipulated to theorize that any organ, in this case intestinal epithelium, may secrete its own circulating growth inhibitor. Resection of a portion of gut would lower the concentration of inhibitor by decreasing the amount produced. As the concentration of inhibitor fell, the remaining gut would begin to grow until sufficient mass was restored to raise the inhibitor concentration to a normal level, at which point growth would cease.

Finally, Tilson and Wright (10) developed the "functional demand theory" in 1970 after demonstrating adaptation responses in bypassed segments of bowel: they reasoned that the consequences of resection resulted in functional alterations that in some way triggered systemically a response that could account for the adaptation witnessed in the bypassed bowel segments.

As will be elaborated in the discussion, much of this recent research can only be explained by considerations of multiple components and stimuli controlling the adaptation response, particularly both the systemic factor evoked by functional demand as well as the local factors contributed by intraluminal contents.

C. Large Bowel Adaptation and Clinical Correlations

Most of the research on intestinal adaptation has been focused upon the response in the small bowel because of the more biologically critical requirements for this organ. However, recently the advances that have been made towards understanding small bowel adaptation have established a framework and foundation for furthering the investigation of intestinal adaptation by examining the responses manifested in the large bowel following intestinal resections.

The intact colon is capable of absorbing up to 2.5 liters of water and 400-600 mequiv. of sodium per day, which is an amount 3-4 times that released from an ileostomy; thus the colon has a considerable reserve capacity.

Wright (11) has reviewed the functional consequences of colectomy with an emphasis upon clinical correlations that merit further research into colonic adaptation. Though almost 90% of all fluid and electrolytes ingested or secreted into the upper gastrointestinal tract has already been absorbed before reaching the terminal ileum, the remaining 10% can cause clinical problems for colectomized patients. Patients with ileostomies excrete water and electrolytes in direct proportion to intake and at rates 7-10 times greater than normal patients on the same diet. Although these losses do not approach normal water and sodium intake, they do obligate the ileostomy

patient to almost daily ingestion of salt and water to avoid extracellular fluid volume depletion as well as susceptibility to formation of renal stones. Also, when sodium intake is greatly increased, the ileostomy patient is forced to increase water excretion in direct proportion to sodium intake.

While adaptation in the small bowel occurs following total colectomy which is manifested by hypertrophy and increased functional ability to absorb salt and water, and in some patients eventually results in the ability to eject an almost solid effluent from their ileostomies, hemicolectomies have not resulted in an adequate adaptive response, leaving the patient with inconvenient management problems of frequently disposing the watery dejecta of their colostomies. It remains to be learned the physical or chemical processes in normal man that converts the fecal stream to solid stool: while water content determines stool volume, its percentage concentration in normal stool and in diarrheal stool is almost the same, suggesting that an unknown mechanism in addition to bowel absorptive capacity is partly responsible for fecal consistency.

In addition to providing information on absorptive problems in colectomized patients, colonic adaptation research is also merited to improve the clinical use of colonic esophageal interpositions (12), not only in terms of deciding which segment of the colon may be utilized with the least functional consequences, but also in terms of understanding how the interposed colonic segment may function and adapt.

Finally, research on colonic functional and morphological adaptation may serve as a useful model to advance the basic science knowledge of epithelial physiology: transport mechanisms, hormonal and pharmacological mechanisms, basic biological adaptation mechanisms, etc. What is learned about colonic adaptation may illuminate and contribute to the understanding of small bowel adaptation or kidney adaptation, and thereby also contribute indirectly to the clinical correlations of life threatening small bowel disease or renal disease.

D. Design of Experimental Problem

After review of the research performed which examined intestinal adaptation in the small bowel in terms of morphology and function, it was decided to design an experiment to correlate the adaptive changes in the remaining colon resulting from hemicolectomy. In addition, the experiment was designed to test some of the possible mechanisms responsible for colonic adaptation, and to compare these results with the data accumulated relating to the various theories of small bowel adaptation.

It was hypothesized that colonic adaptation following hemicolectomy occurred in a manner similar to small bowel adaptation according to Tilson and Wright's "Functional Demand Theory" (10), i.e., that resection of bowel results in a deficiency state, which in turn triggers a hormonal factor systemically acting to establish compensatory

adaptation to restore the deficient state, and thereafter relieve the stimulus for hormonally mediated further adaptation.

In the case of hemicolectomy, it was hypothesized that the post-operative course of diarrhea constituted a relative increase in salt and water loss (in proportion to loss of salt and water absorptive functioning of resected colonic epithelia), resulting in a relatively salt deficit status in the rat, leading to an activation of a systemically mediated hormonal factor effecting compensatory colonic adaptation in salt and water absorptive functioning in the remaining colon to restore the Na metabolism to a normal level.

To test this hypothesis, one group of control rats would undergo left colectomies followed by normal diets and water consumption, and adaptation parameters would be observed in the remaining right colons. Another group of rats would also undergo left colectomies, but post-operatively would be salt loaded with a .45% normal saline fluid restriction as an experimental method for aborting the salt deficiency state assumed to exist in the control group of rats.

In this manner, several hypothesized objectives could be tested:

1. Would left colectomy in the rat under normal circumstances of water diets result in compensatory adaptation of the right colon; if so, since the left colectomy is "downstream" from the sight of adaptation response, it would be apparent that the adaptation response could not exclusively be mediated

by intraluminal contents (whether by dietary bulk, intraluminal nutrition, or endogenous hormones from within the gastrointestinal tract), which presumably would remain unchanged "upstream" in the right colons, but rather must be mediated by some systemic factor.

2. Would salt loading left colectomized rats eliminate a salt deficiency state and thereby abolish or preclude the initiation of the systemic mechanism employed in establishing colonic adaptation, resulting in a failure of the right colons to adapt morphologically or functionally; if so, it would become apparent that not only was a systemic hormonal factor required for colonic adaptation, but also that the mechanism of this factor would be dependent in some manner upon the balance of Na metabolism.

3. Furthermore, if salt-loaded left colectomized rats failed to demonstrate compensatory adaptation in the right colons, while water-fed rats did demonstrate this adaptation, it could be concluded that neither the mechanisms of neuro-vascular factors or tissue mass factors could play a predominant role, since both groups of left colectomized rats would anatomically present with the same post operative neuro-vascular and tissue mass status, unless these factors in some way were also mediated by the vicisittudes of Na metabolism, which is not directly a likely possibility.

The experiment design allowed adaptation response to be measured morphologically in terms of evidence of hypertrophy and/or hyperplasia, and functionally in terms of alterations in the parameters of salt and water absorptive capacity, transmural potential difference, and Na-K- ATPase activity.

METHODS

A. Objectives

1. Experimental Groups:

The objectives of the experiment were to quantitatively identify any significant differences in the morphological and functional adaptive changes in the right colon following left colectomies. It was also decided to determine whether salt loading might affect the mechanisms by which these adaptive changes may take place, and whether the adaptive response is related in part to sodium balance during the post operative period.

The following groups of Sprague-Dawley male rats were defined:

Group A Water Rats: rats with left colectomies postoperatively given normal solid food diets with water.

Group B Salt Rats: rats with left colectomies postoperatively given normal solid food diets but with fluid intake restricted to .45% normal saline.

Group C Control Rats: control rats (no operation) with normal diets with water.

Group D Sham Rats: sham control rats postoperatively given normal diets with water.

2. Adaptation Parameters:

The parameters of adaptation measured were:

(1.) Morphological: hypertrophy and hyperplasia

(2.) Functional: Na-K-ATPase activity, salt and water resorptive capacity, and transmural potential differences (P.D.).

B. Surgery and Preparation of Experimental Groups

The above morphological and functional adaptation parameters were measured in each group of rats in the following manners:

Group A Water Rats 17 Sprague-Dawley male rats weighing between 170 and 242 grams were prepared for surgery by restriction to a pure water diet for 24 hours. They were then anesthetized with an injection of Pentobarbital into the peritoneum at a dose of 40 mg/kg. Each rat was secured on its back on a surgical board, and a midline abdominal incision was performed from 1 cm. below the sternum to 1 cm. above the anus. The peritoneum was opened and the small bowel was lifted and placed laterally to the right on gauze pads soaked in saline. The left colon was identified and pulled laterally and placed on the left on a wet gauze pad, and the inferior mesenteric vessel branches leading to the distal colon were identified and ligated with silk, leaving intact the vasculature to the rectum. The mesentery was then dissected bluntly from the descending colon proximally until branches of the superior mesenteric vessels feeding the area of the proximal side of the splenic flexure were identified. Proper identification of these vessels required minimal sharp dissection of mesentery; these vessels were then ligated with silk at about ½ cm. proximal to their insertion into colonic mucosa, and the remainder of the left colon, including the splenic flexure, was freed from mesentery.

The left colon was then resected proximally at the mid-transverse region and distally at a site 1 cm. proximal to the rectum. Any remaining fecal contents in the proximal half of the transverse colon were manually expressed from the colon onto the gauze. The abdominal cavity and viscera were then irrigated with saline, and a mid-transverse-rectal end to end anastomosis was performed using 5-0 Dexon sutures. After saline irrigation, the viscera were reinserted into the abdominal cavity, and the abdominal wall was closed in two layers with 3-0 Dexon.

Post-operatively, these rats were given a standard food pellet diet with distilled water, and weighed weekly for a period of 6 weeks, after which adaptation parameters were measured, as discussed below.

7 of the 17 rats died in the first week, presumably a result of inexperienced surgical technique; however, the remaining 10 rats recovered satisfactorily, as evidenced by weight gaining measured weekly (see Figure 1, on page 30).

Group B Salt Rats 15 Sprague-Dawley male rats had left colectomies performed exactly in the same manner as for the Group A Water Rats. However, postoperatively, these rats were restricted to a .45% normal saline fluid intake, while receiving the same standard food pellets as the Group A Water Rats. These rats were also weighed weekly for a period of 6 weeks, with only 1 rat dying in the 3rd week. The remaining 14 rats recovered satisfactorily, as evidenced by their weight gain summarized in Fig. 1.

While the growth curves for the salt-rats were slightly higher than for the water-rats, indicating perhaps a systemic factor mildly limiting recovery and growth through a presumed salt-depletion mechanism not affected by the salt-loaded rats, the differences in growth rates between the two groups at the end of the 6 week post operative period was not significant.

After 6 weeks, adaptation parameters were measured on the Group B Salt Rats as discussed below.

Group C Control Rats At the end of the six week period, 10 control rats were purchased for measurement of adaptation parameters. These rats had been on a standard food pellet diet with water, were retired breeders, and slightly older than the rats purchased for the Groups A and B Rats, weighing an average of 552 grams/rat, vs. 418 grams/Group A Water Rat and 429 grams/Group B Salt Rat. however, it was felt that by the end of the 6 week period, the Groups A and B Rats had matured sufficiently to be comparable to these control rats.

Group D Sham Rats 10 Sprague-Dawley male rats in a previous experiment underwent sham operations in which the abdomens were opened and mid-transverse colons transected and immediately re-anastomosed end to end without loss of significant colonic tissue. After 6 weeks, these rats also underwent some of the adaptation parameter measurements discussed below.

C. Measurement of Adaptation Parameters

Adaptation parameters were measured in the Group A Water Rats, Group B Salt Rats, and Group C Control Rats within a one week period six weeks following the surgery performed. The adaptation parameters of the Group D Sham Rats had been measured in a previous experiment, but also at a time six weeks following the sham operation.

1. Morphological Adaptation:

Evidence for hypertrophy and/or hyperplasia of the colonic mucosa as a manifestation of morphological adaptation was based upon three observations: indirectly by weighing a segment of the remaining right colon intact, indirectly by weighing the scraped mucosa of the colonic segment, and directly by examination of histologically prepared slides of random biopsy specimens of the colonic segment. The indirect methods of measuring hypertrophy and/or hyperplasia have been utilized successfully by other investigators (13).

The rats were anesthetized with Pentobarbital 40 mg/kg and the abdomen opened as before. The colon was brought into view, and the anastomosis identified in the colectomized rats. Potential difference and perfusion studies were performed as described below. Then a segment of the colon, averaging 10-15 cm. in length, was resected, from 1 cm. distal to the ileocecal valve to 1 cm. proximal to the anastomosis in the colectomized rats, and to a site at the mid-transverse colon in the control rats.

The segments were then rinsed with saline intraluminally and externally to eliminate any fecal or hemorrhagic contents, and all remaining mesentery was bluntly and sharply dissected from the colonic segment. The segment was briefly dried on a dry sponge for two minutes, and then measured in length under a constant 15 gms. of tension. The segment was then weighed intact, and the gut weight was recorded in mg/cm.

A biopsy specimen was then taken from the exact middle of each intestinal segment and placed in a 10% formalin solution for histological preparation, as discussed below.

Following this, the two remaining halves of each colonic segment were incised longitudinally, and the segments stretched flatly on a piece of clean cardboard exposing the mucosal surface. The mucosa was then scraped easily from the serosa with a dulled scalpel. The scraped mucosa was then weighed and recorded as mucosal weight in mg/cm.

Random histological sampling of the residual tissue revealed that the gut scraping in this manner completely removed villus and crypt cells.

A direct observation for morphological changes was performed by examination of the biopsy segments, which were sectioned transversely at 5 microns and stained with hematoxylin-eosin. All sections were coded to prevent biased reading, and were read "blindly" in the microscope, using a micrometer, as follows: the height

of the 10 tallest villi of each biopsy specimen was measured from the base of the crypt to the top of the crypt (as recognized by a slight decoloration of the cells at that point), and the number of cells were counted in a vertical column from the base of the crypt to the top of the villus. These observations were recorded from random biopsy specimans from the Group A Water Rats, Group B Salt Rats, and Group D Sham Rats.

2. Functional Adaptation:

In order to measure parameters of functional adaptation in the right colon following left colectomy, it was decided to observe indicators of the most well known physiological colonic function, i.e., salt and water resorption. In addition to actually measuring the salt and water resorption of the right colon, a Na-K-ATPase assay was developed and potential differences were measured from lumen to serosa, factors which theoretically should parallel the direction of altered resorptive capacity. In this manner, three sets of observations were made to consistently assess the relative magnitude of the functional resorptive capacity of the right colon as determined by left colectomy as well as by salt loading.

Na-K-ATPase Activity Na-K-ATPase activity of the scraped mucosa of the right colon was measured by using a variation of the method of Katz and Epstein (14), whereby essentially a homogenate of the colonic mucosa was prepared, ATP added (with which the Na-K-ATPase would liberate Pi), the amount of Pi liberated measured quanti-

quantity of protein measured by the method of Lowry (16), and the Na-K-ATPase activity recorded as a function of the uM Pi/mg protein/hr. Details of the assay were as follows:

(a) Preparation of Fresh Homogenate: after the right colonic segments had been weighed for measurement of the morphological parameters, the mucosal scrapings of each rat were placed into a chilled vial and a homogenizing solution was added in a 1/20 w/v ratio. The homogenizing solution was prepared daily by mixing 2 solutions in a 9:1 ratio, the first solution consisting of sucrose .25M, imidazole 30mM/liter, and EDTA 5mM/liter, which had been brought to a pH of 6.8, and the second solution consisting of 1% Na deoxycholate. The combined solutions were then brought to a pH of 6.8 (pH adjustments were made using either HCl or Tris). The tissue-solution was then homogenized in an ice bucket with a Sorvall micro-homogenizer for 5 minutes, and the resulting suspension was filtered through a double layer of gauze, resulting in a fresh whole homogenate of colonic mucosa. 1-2 ml. of this homogenate was placed in a marked vial and refrigerated for subsequent determination of protein content (see below), while the remaining fresh homogenate was immediately utilized for Pi measurements.

(b) Incubation Reaction of Fresh Homogenate with ATP: Two incubation media were prepared, one containing 100 mM NaCl/liter, 20 mM KCl/liter, and 10 mM imidazole/liter (brought to a pH of 7.8), and the other media containing

120mM NaCl/liter and 10 mM imidazole/liter (also brought to a pH of 7.8). Just before the reaction, an ATP solution was prepared consisting of 10 units of previously prepared and frozen 100 mM/liter Na_2ATP and 1 unit of 1M MgCl, and this solution was brought to a pH of 7.8. Duplicate Erlenmeyer flasks were then prepared for each incubation media, the first set containing .2 ml. of fresh homogenate, 4.0 ml. of the incubation media containing K+, and .4 ml. of the ATP solution; the second set of duplicate flasks containing the same amounts of tissue and ATP, but incubated with 4.0 ml. of the media that did not contain K+. In this manner, because Na-K-ATPase requires both Na and K, the first set of duplicates would include liberation of Pi from ATP as a result of Na-K-ATPase, while the second set would not; hence, the net difference of measured Pi would relate exclusively to the Na-K-ATPase activity. Also, the Pi liberated by other ATPase in the tissue (eg. Mg-ATPase)would be netted out equally in both incubation reactions.

It should be pointed out that duplicates of blank flasks, containing neither tissue nor ATP, were also incubated and measured subsequently for Pi contamination, and these blanks in all cases revealed no significant Pi contamination calorimetrically when Pi concentration was subsequently measured.

Finally, duplicate flasks containing incubation media and ATP, but no tissue, were incubated to get a

measurement of the spontaneous breakdown of ATP to Pi; and in all cases, the Pi generated in this manner was found to be calorimetrically insignificant, and, in any case, equally netted out from the comparison of the incubation media containing K^+ with that not containing K^+.

All incubation reactions took place in a shaking warm water bath (37° C.) for exactly 15 minutes, each set being sequentially timed.

Following 15 minutes of reaction, 2 ml. of 35% trichloroacetic acid (TCA) was added to each Erlenmeyer flask to precipitate the protein, and the flasks were immediately shaken and placed in an ice bath to stop the enzymatic reactions.

The flasks were then centrifuged at 2800 rpm for 10 minutes, and the supernatent filtered into marked test tubes on ice.

(c) Fiske and Subbarow Determination of Pi: To determine the amount of Pi in the supernatent that had been liberated from ATP by the ATPase enzymes in the tissue, the method of Fiske and Subbarow was employed. A solution of 2.5% ammonium molybdate mixed with 5N H_2SO_4 was prepared, and a reducing reagent prepared as a 100 ml. solution containing 15% Na bisulfite (anhydrous), 0.25 gm. 1-amino-2-napthol-4 sulfonic acid (ANSA), and o.5 gm. Na sulfite (anhydrous). The reducing reagent was mixed vigorously for 15 minutes, filtered, and stored in a dark brown bottle in the refrigerator. The reaction was carried out as follows: 1.0 ml. of the above supernatent (tissue) was added to 3.0 ml. of demineralized

water and to 0.4 ml. of the molybdate solution, mixed, and then o.2 ml. of the reducing reagent (ANSA) was added, and the tubes mixed over a 10 minute period. The reaction essentially consisted of the reduction of a molybdate-Pi complex, which contributed a blue coloration that became visible, the intensity of the blue being proportionate to the concentration of the Pi in the sample. Following the 10 minute reaction, each tube was read in a double barrel spectrophotometer set at 660 mu, and absorbance readings were recorded as a function of the blue coloration calorimetrically.

In order to convert the absorbance readings with the Pi concentrations, Pi standards were prepared from $K_2HPO_4 \cdot 3H_2O$ in concentrations of .4, .8, 1.6, and 2.4 uM Pi/ml. The standards of Pi underwent reactions for 10 minutes as described above, except that 1.0 ml. of each standard was substituted for the 1.0 ml. of tissue specimen. The standards were then read on the spectrophotometer, and the absorbance readings were plotted on a graph as they related to the known Pi concentrations. It was found that the concentrations of Pi correlated with the absorbance readings in a linear relationship.

(d) Determination of Protein Content: The method of Lowry was used. Each day, a solution of 2% Na_2CO_3 in 0.10N. NaOH was added to a solution of 0.5% $CuSO_4 \cdot 5H_2O$ in 1% Na tartrate in a 50:1 ratio. 5.0ml. of this combined reagent was then added to 1 ml. H_2O and to this was added 0.02 ml. of tissue from the original fresh

to react for 10 minutes, followed by the addition of 0.5 ml. of Folin-Ciocalteu phenol reagent diluted to 1.0 N. acid, mixing, and allowing 30 minutes for the reaction. A blue coloration appeared as a function of protein content, and absorbance was read from the spectrophotometer set at 600 mμ.

Standards of bovine serum albumin in the concentrations of 1, 2, 4, 6, 8, and 10 mg/ml were prepared, run through the calorimetric reaction, read on the spectrophotometer, and plotted on a graph, resulting in a linear relationship between protein concentration and absorbance.

(e) Calculation of Na-K-ATPase Activity: Pi determinations were made in duplicates with and without K^+ added to the media for each specimen, thus determining the net difference of Pi concentration between the 2 sets of reactions with each tissue specimen as a function of specific Na-K-ATPase enzyme activity. In order to relate the Pi concentration with the protein concentration, it was necessary to multiply the Pi concentration by a factor of 33, because the fresh homogenate used for Pi determination was diluted by a factor of 33 in its processing (0.2 ml tissue divided by 0.2 ml tissue, 4.0 ml H_2O, 0.4 ml ATP, and 2.0 ml TCA). In this manner, Pi concentration was corrected for dilution factors, and Pi and protein concentrations could be compared as representative concentrations from the fresh whole homogenate of tissue. Since the activity of the Na-K-ATPase ezyme is also measured in units of hours, and the actual reaction time

was 15 mimutes, it was also necessary to multiply the numerator by a factor of 4. In this manner, Na-K-ATPase enzyme activity was recorded as uM Pi/mg protein/hour.

It must be acknowledged that the first source of ATP used in the assay had been found in the lab without knowing how old, and consequently how broken down the ATP was; as a consequence, the first run of samples through the assay was not successful, and the assay had to be repeated with a fresh supply of ATP, utilizing the same fresh homogenate of the tissue specimens that had been reserved for such contingencies in the freezer compartment of a refrigerator. 14 rats had undergone the initial assay before the results were obtained and found to be unsatisfactory, and hence assays on these rats were repeated with the fresh ATP after 2 days of refrigeration. The remaining 20 rat preparations were done correctly with the fresh ATP. Of the 14 repeated assays, 10 were consistent with the results of the other 20 rats that had originally been assayed with the fresh ATP, but 4 of the values were so inconsistently erratic from all the other assays that they were not compiled in determining the results.

Salt and Water Resorptive Capacity

Salt and water resorptive capacity was measured in the right colons by perfusion studies employing the method of Levitan (17). In this experiment, only water resorption was measured, and it was assumed that salt

resorption would correlate directly as a function of the water resorption. In previous experiments, Na resorption was determined by measuring the Na content of perfusate on an Auto-Analzer, and found to correlate directly with the measured water resorption.

After the right colons were exposed, an incision was made at the distal end of the cecum and a polyvinyl catheter inserted into the lumen and fastened tightly with a ligature, so that no proximal fluids could enter the perfused segment. Previous laboratory experience had indicated that a more proximal location in the cecum resulted in constant clogging of the catheter by mucous.

Similarly, an incision was made at 1 cm. proximal to the anastomosis of the left colectomized rats and a catheter inserted and tied so that the perfusate would not leak distally. A perfusion solution, consisting of 140 mequiv. of NaCl, 180 mg. glucose/100 ml, and 1% PEG (polyethelene glycol) was then pumped into the proximal catheter with an automatic pump at a constant rate of 0.2 ml/min., and collected in a petri dish from the distal catheter. After 45 minutes of perfusion to establish an equilibration, three 20 minute consecutive samples of perfusate were collected, to be analyzed in duplicates for PEG concentration.

For each sample, 1 ml. of perfusate was reacted with a media of 1 ml. 10% $BaCl_2$, 2 ml. .3N. $Ba(OH)_2$, 2 ml. 5% $ZnSO_4$, and 10 ml. H_2O for 10 minutes in a 37^0 C. water bath. The precipitate was then filtered, and 1

ml. of filtrate was added to 3 ml. of gum arabic solution (6 mg./500 ml.) and 4 ml. of T.C.A. solution (16.5 gm. $BaCl_2$ anhydrous plus 90 gm. trichloroacetic acid plus 300 ml. H_2O, mixed and filtered), and allowed to stand for 1 hour after shaking. The samples were then read on a spectrophotometer set at 650 mu.

Standards of PEG concentrations were prepared and read on the spectrophotometer and plotted on a graph, resulting in a linear relationship. From this graph, the PEG concentrations of the perfusate samples as well as from control samples of the perfusion solution were determined, and water flux calculated as follows:

$$\text{Water Flux} = \frac{V_{in} - V_{in}\left(\frac{PEG_{in}}{PEG_{out}}\right)}{\text{gm. dry intestinal weight}}$$

V_{in} = rate of perfusion = 0.2 ml/min.

PEG_{in} = concentration of PEG in perfusion solution before perfusion of colon

PEG_{out} = concentration of PEG in perfusate after perfusion of colon

Positive values were indicative of net water resorption while negative values indicated net water secretion.

Perfusion studies were performed in a previous experiment on a group of Sham Control Rats 6 weeks following the sham operation in the exact manner as described above.

Transmural Potential Difference

The transmural potential difference (PD) was measured in the right colons of rats as soon as the colons were exposed. The technique was adapted from the method of Edmonds (18). A reference electrode, containing a silver-silver chloride junction was attached to a segment of polyvinyl tubing filled with 150 mequiv. NaCl/agar 4% and inserted into the abdomen in a manner whereby the saline-agar made direct contact with the peritoneum. It is known that the potential difference between gut lumen and serosa is identical to the PD between gut lumen and peritoneum (19). A calomel electrode was also attached to a segment of polyvinyl tubing and filled with 3 M. KCl/agar 4%, and placed intraluminally into the colon at different sites in such a manner as to insure direct contact of the luminal mucosa with the KCl/agar.

The two electrodes were connected to a battery operated high-input impedance millivoltmeter. Initially, the two electrodes were placed into a beaker of normal saline, and the asymmetry of PD was corrected to a zero value. The reference electrode was then placed intra-peritoneally, and the calomel electrode placed 2 cm into the rectum, 2 cm proximal to the anastomosis site of the left colectomized rats via a small incision (distal right colon PD), at a mid-transverse location in control rats, and 2 cm distal to the cecum (proximal right colon PD),

respectively. A PD reading was recorded at each location after stabilization, which usually occurred in 4-5 minutes. The electrodes were washed in saline and corrected for asymmetry before each reading at a new location.

RESULTS

A. Morphological Adaptation

Figures 2 and 3 summarize the indirect evidence of morphological adaptation manifested by hypertrophy and/or hyperplasia in terms of gut and mucosal weight per cm of colon, and indicate that there was no significant differences between the Group A Water Rats and the Group B Salt Rats, nor was there a significant difference between the left colectomized rats (Groups A and B) and the control rats. Thus, this indirect method of measuring gross morphological changes did not reveal detectable evidence of hypertrophy and/or hyperplasia following left colectomy, regardless of salt loading.

Figures 4 and 5 summarize the more direct evidence of histological examination of biopsy specimens for detection of hypertrophy and/or hyperplasia. There was no significant evidence of morphological changes among the three groups in terms of crypt height or number of cells from the base of the crypts to the tops of the villi, except for the difference in number of cells between the Group B Salt Rats and the Group D Sham Rats, which as an isolated finding is hardly documentary evidence that salt-loading left colectomized rats will result in significant morphological adaptive changes; however, additional experiments would be necessary to confirm this apparent discrepancy.

B. Functional Adaptation

Na-K-ATPase Activity: Fig. 6 summarizes the results of the Na-K-ATPase assay, and indicates that there was a significant increase in the Na-K-ATPase activity in the Group A Water Rats in response to left colectomy over both the Group B Salt Rats and the Control kats. Of note is that there was no significant increase in Na-K-ATPase enzyme activity as a result of left colectomy in the salt-loaded Group B Rats over the Controls, thereby suggesting that salt loading after left colectomy somehow abolished the normal functional adaptive response seen in left colectomized rats given a normal diet with water.

Salt and Water Resorptive Capacity: Fig. 7 summarizes the results of perfusion studies that measured the resorptive capacities of the right colons following left colectomies compared to Sham Controls. It was assumed that salt resorption correlated directly with the measured net water resorption.

Salt and water resorption, as a parameter of functional adaptation, was significantly greater in the water fed left colectomized ratsover both the sham controls and the salt-loaded left colectomized rats. However, salt and water resorption in the salt-loaded left colectomized rats were not significantly different from that of sham control rats, indicating that salt-loading may have abolished the normal adaptive response of the right colon to significantly increase its salt and water resorptive capacities

to compensate for loss of resorptive capacities after left colectomies.

It must be acknowledged that apparent laboratory errors in the perfusion studies necessitated the elimination of some of the samples from the compiled results summarized in Fig. 7. Of the 10 Group A Water Rats, 5 of the 10 values were eliminated and of the Group B Salt Rats, 8 of the 14 values were eliminated. Of the 13 eliminations, 1 was due to the rat being used for a Na-K-ATPase assay trial, 7 were eliminated because the values were negative, indicating net secretion of water, which does not coincide with known normal colonic function, and 5 were eliminated because of values that were higher than could be possible at the perfusion rate of 0.2 ml/min. over a 20 minute period. Of note is that the laboratory technician performed the PEG assays in sets of 4 rats, and the above 12 erroneous values were all done in 3 sets of PEG assays; thus, 3 sets of PEG assays were eliminated because of laboratory technique errors, presumably related to inaccurate timing of reactions before reading absorbance in the spectrophotometer, or to inaccurate concentrations and dilutions of reaction reagents.

It was felt that the remaining 3 sets of assays, measuring water resorption in 5 Group A Water Rats and 6 Group B Salt Rats, were reasonable values consistent with several perfusion studies performed on rat colons

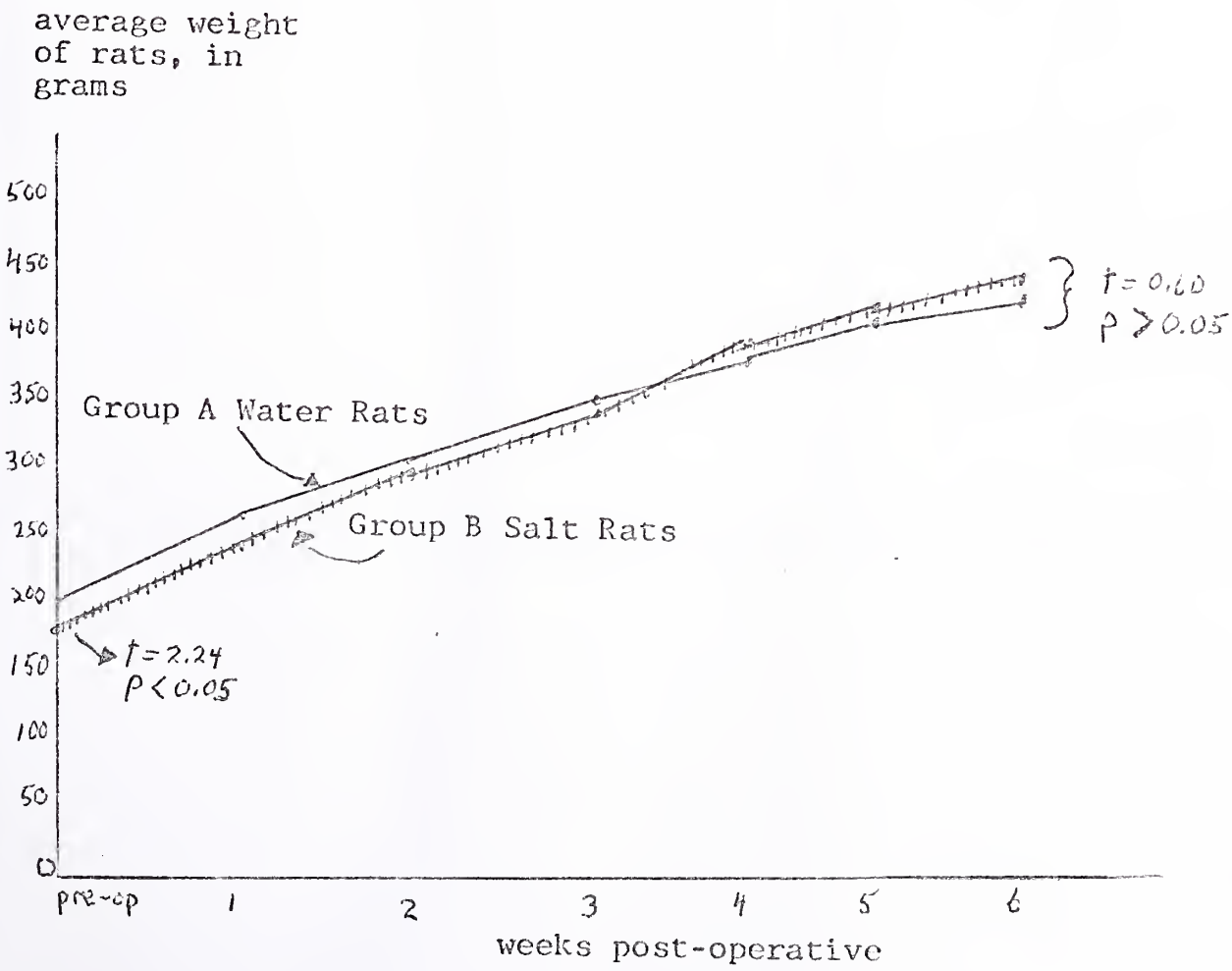
in several previous experiments in the same lab, and would serve to present significant data on salt and water absorption as parameters of functional adaptation of the right colon following left colectomy. Fortunately, other parameters of adaptation relating directly to the function of salt and water resorption, i.e. Na-K-ATPase activity and transmural potential differences, have been measured in this experiment to corroborate the consistency of results in all three parameters of functional adaptation.

Transmural Potential Diffence: Fig. 8 summarizes the transmural PD at the proximal and distal right colonic sites. In the left colectomized rats, the distal right colon PD was greater than the proximal right colon PD.

The proximal and distal PD's of water fed left colectomized rats were significantly greater than both salt loaded left colectomized rats and control rats, while the PD of salt-loaded left colectomized rats was not significantly altered from that of control rats.

Of the 34 total rats, PD's were not measured in 11 rats, 3 of which were sacrified for Na-K-ATPase assay trials (2 controls and 1 salt-loaded rat), 1 control ratwho died under anesthesia, and 7 rats whose PD's were erroneously measured after the perfusion studies (1 hour and 45 minutes) had been completed (3 control rats, 2 salt-loaded colectomized rats, and 2 water-fed colectomized rats). Rectal PD readings did not correlate in any significant manner with the proximal and distal PD readings from all 3 groups.

Figure 1. Growth curves for 6 weeks post-left colectomies for Group A Water Rats and Group B Salt Rats. While the average weight of the Group A Water Rats was significantly greater than the average weight of the Group B Salt Rats pre-operatively (coincidentally determined by random assignment of rats to the 2 groups), this difference of weight between the 2 groups lost significance by the sixth post-operative week.



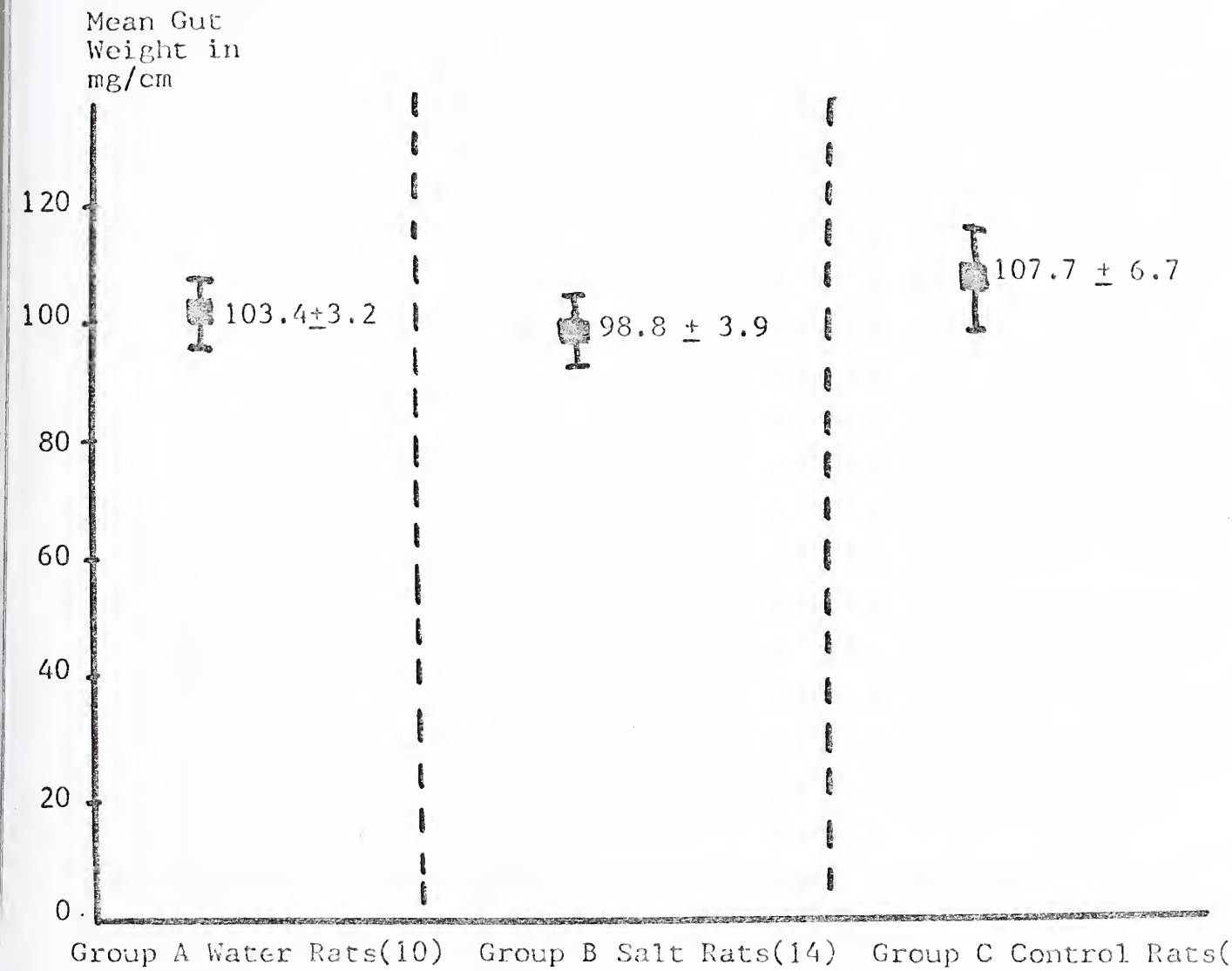


Figure 2: Gut weight per cm. as a manifestation of hypertrophy and/or hyperplasia following left colectomy among water-fed rats, salt loaded rats, and control rats who did not undergo operation. There is no significant difference in gut weight/cm among the 3 groups. Number of rats in parentheses.

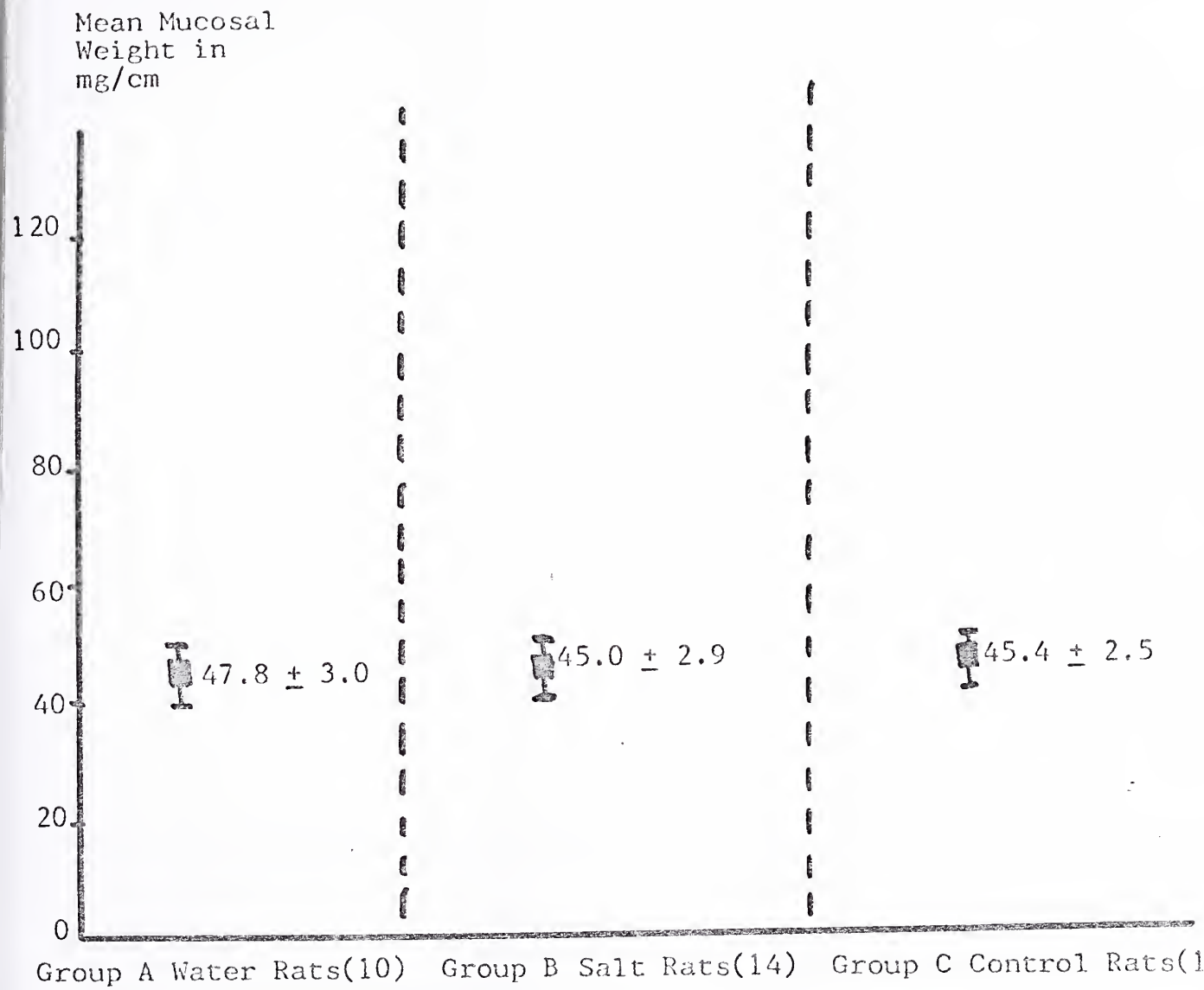


Figure 3: Mucosal weight per cm. as a manifestation of hypertrophy and/or hyperplasia following left colectomy among water-fed rats, salt loaded rats, and control rats who did not undergo operation. There is no significant difference in mucosal weight/cm among the 3 groups. Number of rats in parentheses.

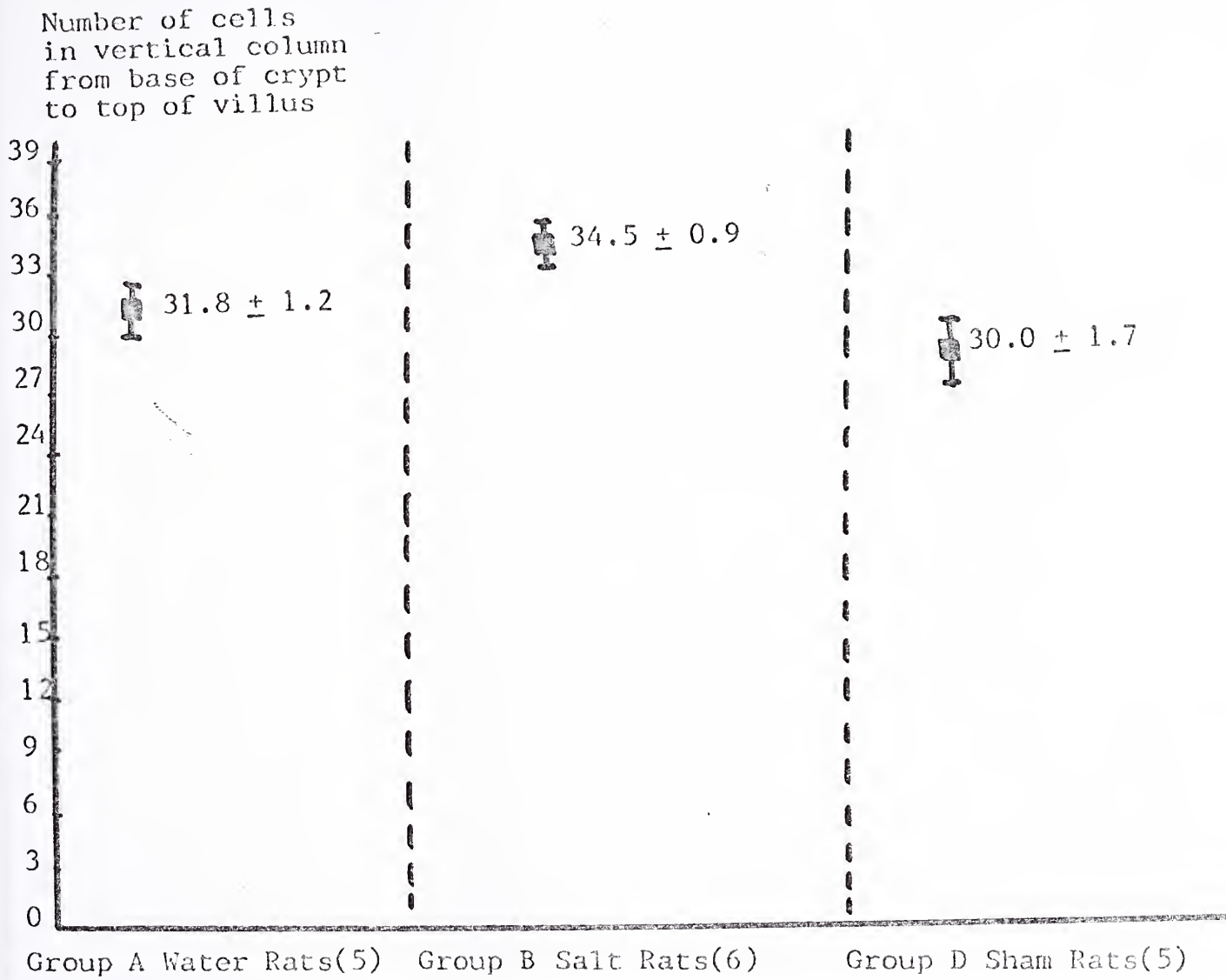


Figure 4: Cell count as manifestation of hypertrophy and/or hyperplasia following left colectomy among Group A Water Rats, Group B Salt Rats, and Group D Sham Rats. There is no significant difference ($p > 0.05$) in number of cells between the Group A Water Rats and Group B Salt Rats or Group D Sham Rats; however, the difference in number of cells between the Group B Salt Rats and the Group D Sham Rats is significant ($p < 0.05$). Number of biopsies examined in parentheses.

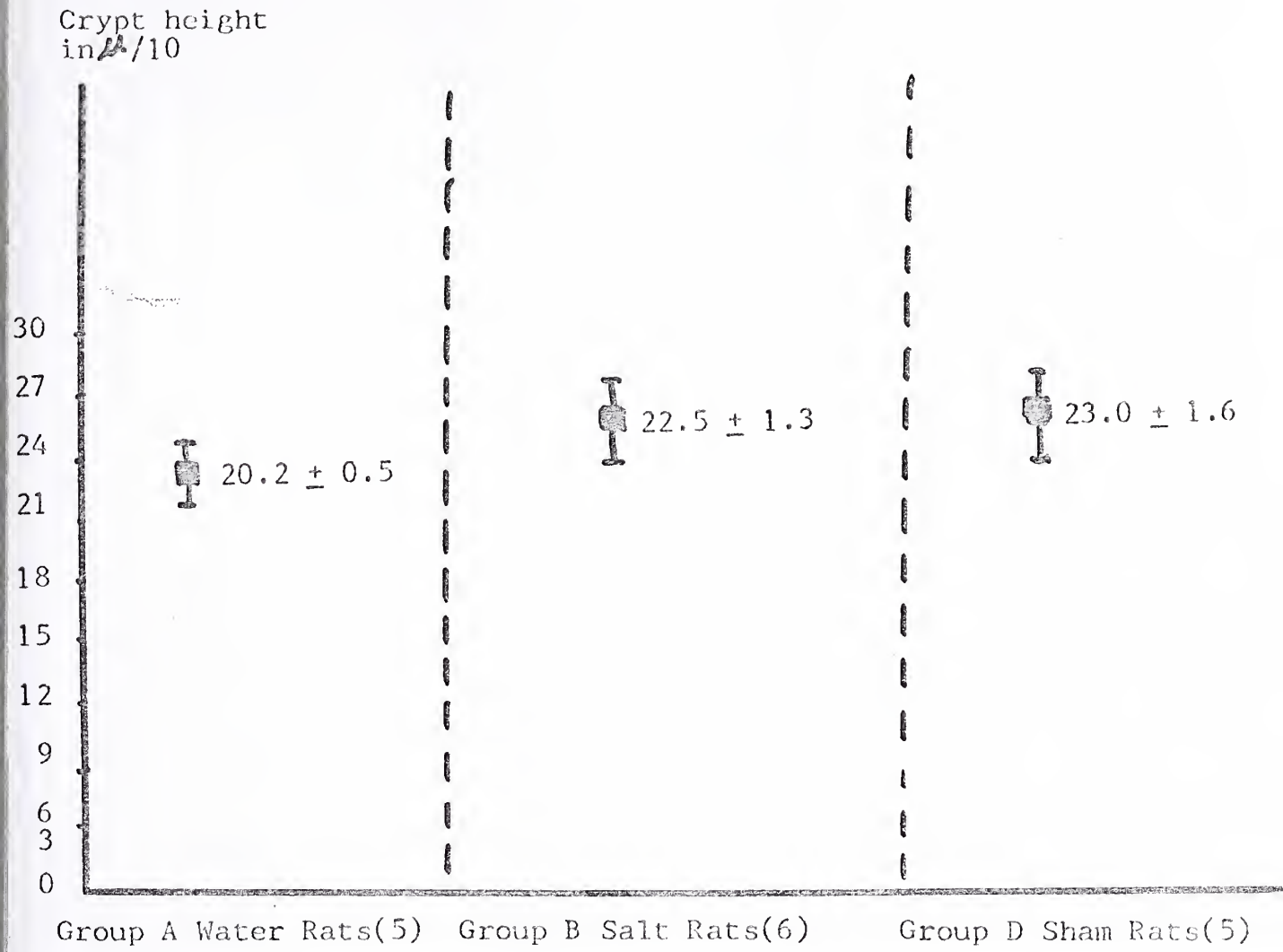
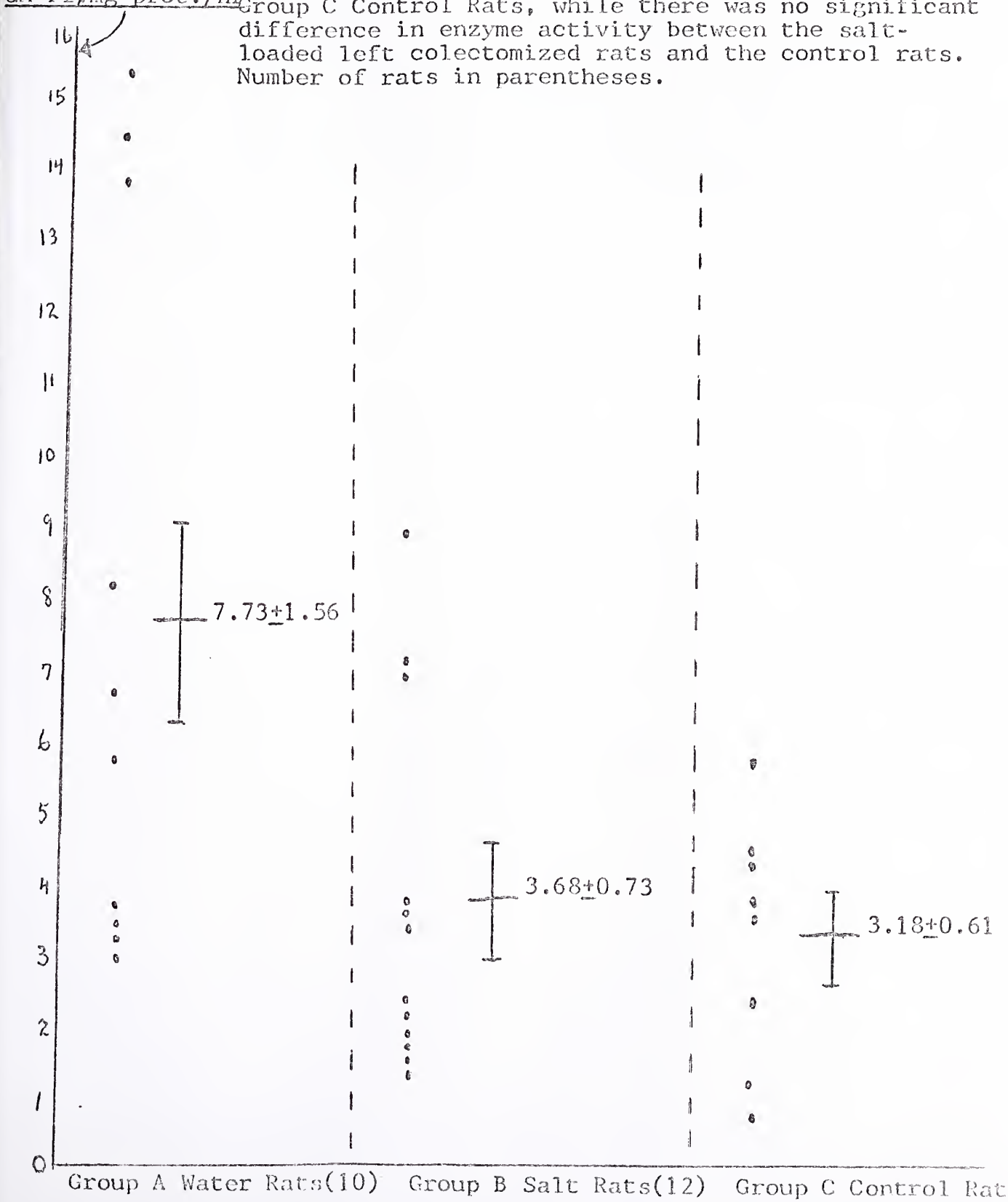


Figure 5: Crypt height as manifestation of hypertrophy and/or hyperplasia following left colectomy among Group A Water Rats, Group B Salt Rats, and Group D Sham Rats. There is no significant differences ($p > 0.05$) in crypt height among the 3 groups. Number of biopsies examined in parentheses.

Figure 6: Na-K-ATPase activity in terms of uM Pi/mg protein/hr. was significantly greater in the Group A Water Rats over both the Group B Salt Rats and the Group C Control Rats, while there was no significant difference in enzyme activity between the salt-loaded left colectomized rats and the control rats. Number of rats in parentheses.



H_2O > Salt, $t = 2.341$; $p < 0.05$ Salt > Control, $t = .527$, $p > 0.05$

H_2O > Control, $t = 2.708$; $p < 0.05$

Figure 7: Salt and Water Resorption: rats with left colectomies fed a normal diet with water adapted functionally in remaining right colon by significantly resorbing more water than either Sham Control Rats or left colectomized rats that were salt-loaded; while there was no significant resorptive change between the salt-loaded left colectomized rats and sham controls. It is assumed that salt resorption correlates with theseresults as an indirect function of the water resorbed. Number of rats in parentheses.

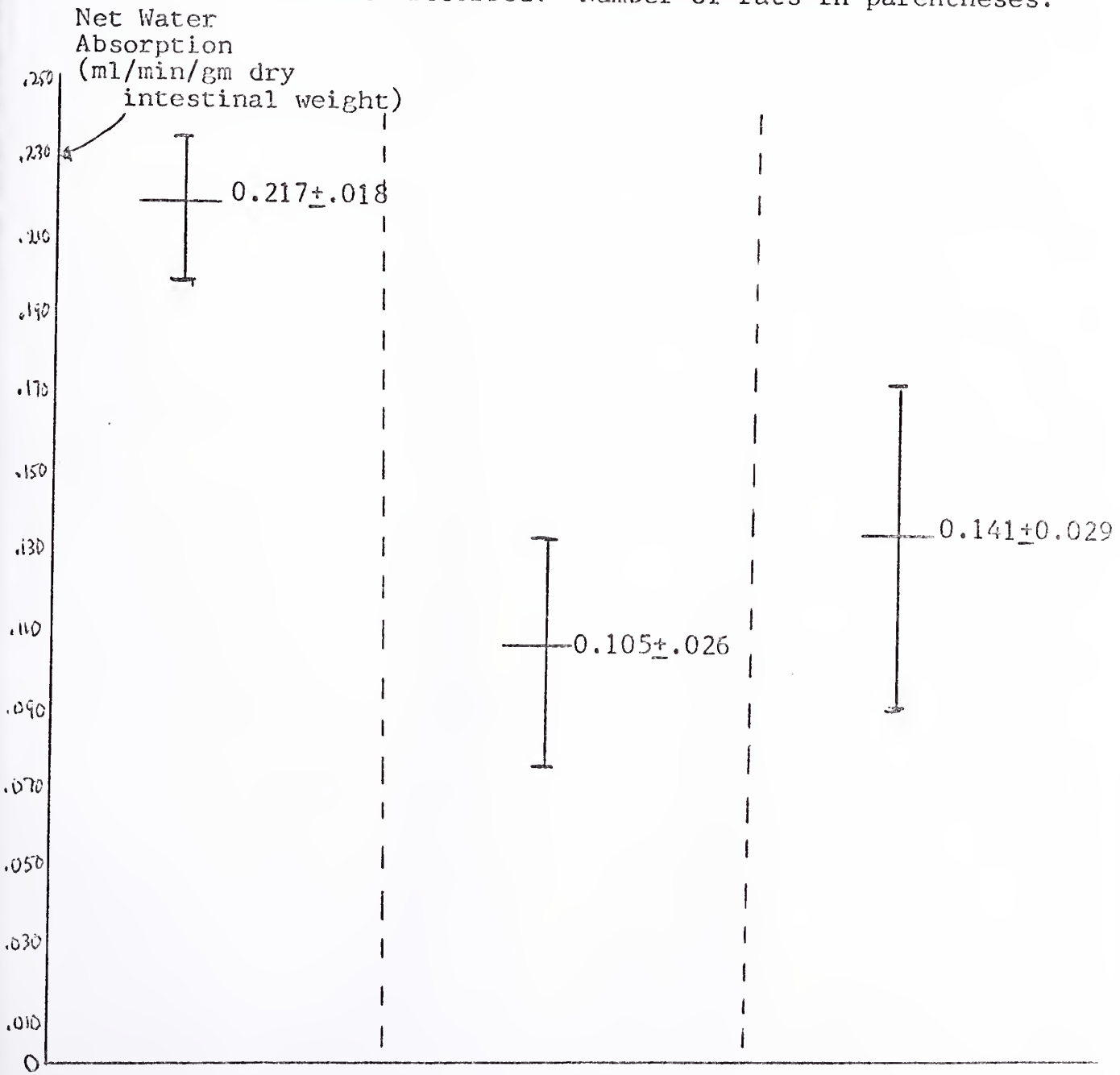
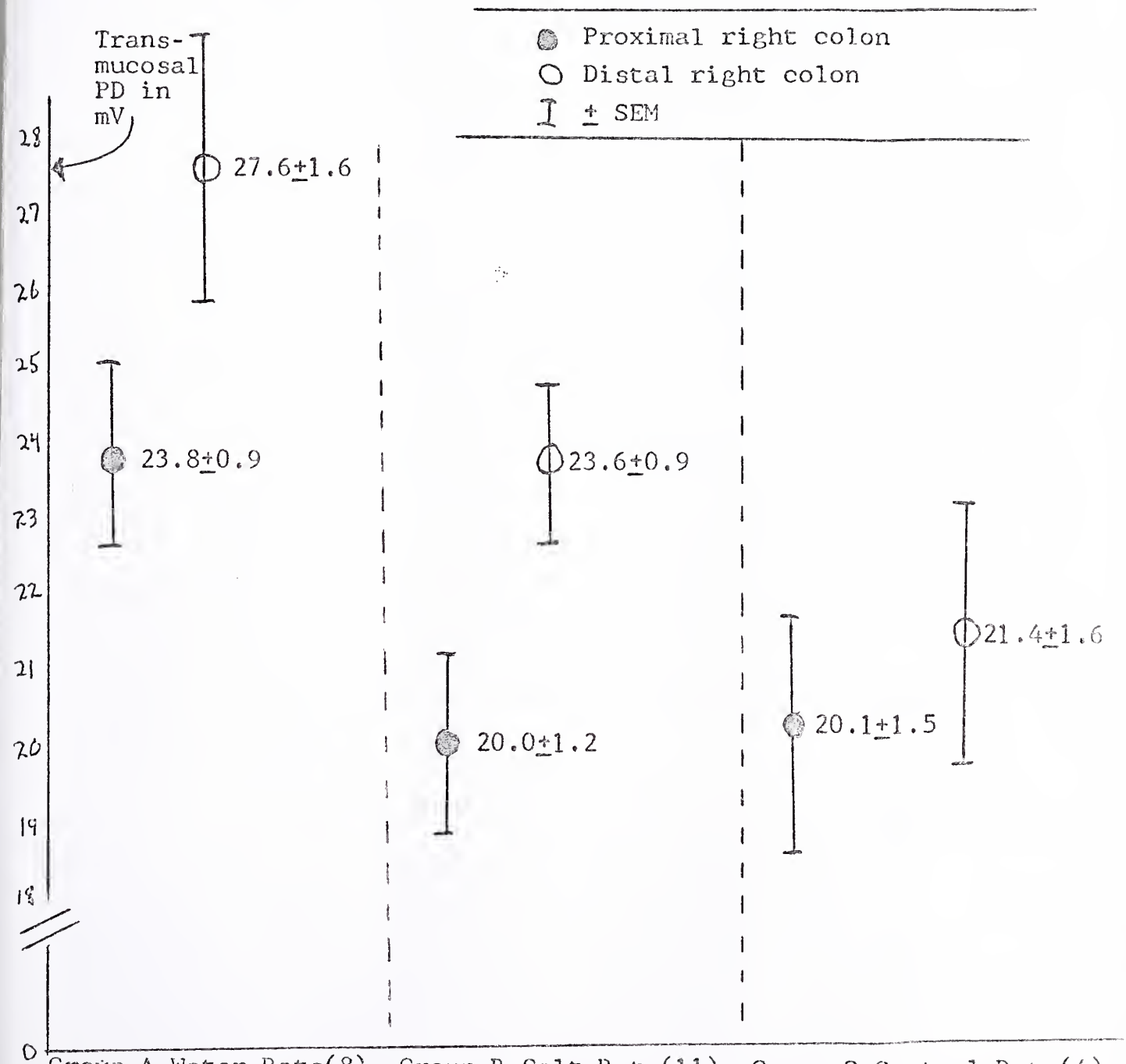


Figure 8: The proximal and distal transmucosal potential difference was significantly greater in the water fed, left colectomized rats over both the salt-loaded left colectomized rats and the control rats, while there was no significant differences in potential difference between the salt-loaded left colectomized rats and the control rats. Number of rats in parentheses.



Proximal PD H_2O > Salt Rats, $t=2.528$; $p<0.05$
Proximal PD H_2O > Control Rats, $t=2.097$; $p<0.05$
Distal PD H_2O > Salt Rats, $t=2.159$; $p<0.05$
Distal PD H_2O > Control Rats, $t=2.698$; $p<0.05$

Proximal PD Salt > Control $t=0.050$; $p>0.05$
Distal PD Salt > Control, $t=1.201$; $p>0.05$

DISCUSSION

A. Discussion of Experimental Results

The experimental results indicated that left colectomy in water fed rats did not result in significantly morphological adaptation in the right colon in terms of gut weight, mucosal weight, cellular hyperplasia, or crypt height when compared to controls. Neither did salt-loading left colectomized rats result in significant morphological adaptation, except for the isolated measurement of cellular hyperplasia, which was increased over controls. This anomaly may have been sporadic due to the relatively insensitivity of the laboratory technique, or may actually reflect a cellular hyperplasia in response to salt loading and an excessive sodium balance, though an interpretation of this response would be puzzling. In any event, salt depletion, if resulting from water-fed left colectomized rats, did not contribute to a significant functional demand to elicit a stimulus evoking morphological adaptation in the right colon of the rat.

In contrast, the experimental results indicate that water-fed left colectomized rats adapt functionally in the right colon manifested by significant increases in Na-K-ATPase activity, salt and water absorptive capacity, and transmural PD, and that these corroborated parameters of functional adaptive stimuli were abolished by salt-loading left colectomized rats.

Therefore, while much of the research on small bowel intestinal changes demonstrated a correlation between morphological and functional characteristics in

adaptation, it appears that such structural-functional correlates do not necessarily apply to the adaptation response in the right colon following left colectomy. Furthermore, because left colectomy is "downstream" and followed by right colon functional adaptive responses, it is unlikely that intraluminal contents play a significant role in the adaptation response manifested in the colon. Finally, because salt loading abolished the adaptive response in the right colon of left colectomized rats, it is suggested that left colectomy results in a relative state of salt depletion, and that this state imposes a functional demand that somehow elicits systemically a hormonal factor resulting in compensatory functional adaptation in the right colon to increase salt absorption in order to meet the functional demand; without the state of salt deficiency (created by salt loading), functional demand is not detected, with the consequence that the initiating stimulus evoking the adaptation response is not triggered and right colon adaptation fails to occur.

In order to appreciate the perspective of these results as they relate to the over all research efforts in intestinal adaptation, a detailed review of the research of control mechanisms of intestinal adaptation will now be presented, followed by a review of the latest insights into physiological, pharmacological, and biochemical mechanisms of intestinal functional adaptation.

B. Review of Control Mechanisms of Intestinal Adaptation

Intraluminal Factors: The intraluminal contents theories considered three factors of intraluminal contents that may contribute to intestinal adaptation following resection. The first factor, described as the "dietary bulk hypothesis", was formulated by Booth et. al. (4) in 1959: they had performed 1/3 and 2/3 resections of either the proximal or distal small bowel and found that proximal resections resulted in hypertrophy of the distal small bowel, but that distal resections did not result in hypertrophy of the proximal bowel. Because of the later results, they concluded that compensatory hypertrophy did not result from loss of intestine per se, but rather must result from other factors. Citing the work of Kennedy and McCance (25), who had shown in 1958 that by bulk-loading the diets of rats with kaolin in a 3:1 ratio, the rat intestines became enlarged by a factor of four, Booth explained his results by suggesting that distal hypertrophy following proximal resection resulted from the increase of chyme delivered to the distal bowel, and that hypertrophy occurred in order to accomodate the larger volume of intestinal contents reaching the distal bowel. Since the volume of chyme reaching the proximal bowel after distal resection was unchanged, this would account for the lack of hypertrophy in the proximal bowel.

However, as early as 1960, Løran and Althausen (21) demonstrated that 10% distal resections did in fact result in significant hypertrophy in the proximal bowel, thereby raising questions to an exclusive role of dietary bulk controlling adaptation.

It had been shown in 1943 that experimentally induced hypothalamic hyperphagia resulted in intestinal hypertrophy in the rat,(22), and Jervis (23) demonstrated similar results by inducing hyperphagia with chronic alloxan diabetes in 1966.

In 1965, Riecken, Dowling, and Booth (24) found that high bulk diets did not result in hypertrophy, but they did find an increase in functional absorptive capacity in the small bowel which was associated with an increase in the concentration of enzymes in the absorptive epithelium. In 1967, Fischer (25) was also able to demonstrate a correlation of functional adaptation dependent upon nutrition by observing an increase in Beta-galactosidase activity in the rat small intestine after feeding a high lactose diet.

Thus, by 1967, Dowling and Booth (5), (26), modified their thinking by concentrating on the influence of intraluminal nutrition upon intestinal adaptation. In that year, they found that after proximal resection, the distal bowel in the rat was not only hypertrophied, but also accompanied by an increase in functional absorptive capacities because glucose transport was increased in the

ileum. In this experiment, they did not detect an increase in the concentration of enzymes per unit area of mucosa, and concluded that the increase in functional absorptive capacity paralled the morphological increase in surface area of adapted mucosa. Furthermore, after ileal-jejunal transposition, they found that the transposed ileum demonstrated marked hypertrophy and increased glucose absorption, while the transposed jejunum failed to reveal adaptive changes over its usual morphological and functional characteristics. Thus, they reasoned that the increased intraluminal nutrition exposed to the transposed ileum accounted for the adaptation stimulus, while the decreased intraluminal nutrition reaching the transposed jejunum would not have provided a stimulus for adaptation.

A third consideration of intraluminal contents examines the possible roles of intraluminal endogeneous hormones secreted by gastric, duodenal, biliary, or pancreatic exocrine glands. In 1970, Altmann and LeBlond (6) sought an explanation for the longitudinal gradient of villis size along the small bowel, whereby villus size decreased progressively along the entire length of the small bowel, being nearly three times as large in the upper duodenum as in the terminal ileum. They inserted ileal segments into the jejunum and found that the villi enlarged to a size comparable to that of the local jejunal villi. Conversely, jejunal segments inserted into the ileum had their villi decrease in size to that of the

local ileal villi. This led them to believe that villus size and ability to adapt (hypertrophy) was influenced by the environment that bathed them, most probably by the different constituents of chyme in the jejunum and ileum. They continued by inserting duodenal segments into the ileum, but found that while in this case the villi did not increase in size the distally located ileal villi became enlarged. Therefore, they concluded that the duodenum produced secretions which not only neutralized the villus reducing effect of the ileal environment, but also exerted a potent villus enlarging effect. They found that pyloric secretions had a similar effect. Thus, they accounted for the longitudinal gradient by concluding that an intermediate sized villus in a chyme-free, non-functional intestine, would normally be modified by two types of factors: a villus enlarging factor present in pyloric and duodenal secretions accounting for increased villus size proximally along the concentration gradient of the secretions, and a villus reducing factor present in the ileal chyme accounting for diminishment of villus size.

However, this theory has been tested by Tilson (27) in 1975, who found that after esophagoileostomy with total diversion of pancreatobiliary secretions to the colon, hypertrophy and hyperplasia was still documented in the ileum, indicating that morphological adaptation may occur without the influence of endogenous intraluminal secretions from gastric, duodenal, biliary, or pancreatic sources.

Mak (28) found that pentagastrin had no influence on cell proliferation kinetics in fed rats, but that after fasting rats for 64 hours, cell proliferation was suppressed, more markedly in colonic crypts than in duodenal crypts; however, it was found that pentagastrin restored the cellular proliferation activity in these starved rats in the colon and duodenum to levels comparable to those of fed rats.

In 1977, Feny (29) prepared jejunoileostomies with bypass of 85% of the small bowel in rats. Another group of rats had the same bypass operation but also diversion of secretions from the duodenum, pancreas, and bile to the transverve colon; while a third group of rats also had gastric secretions in addition to the other above secretions diverted to the transverse colon. In all three groups, hypertrophy developed in both the jejunum and ileum in circuit, though the third group with gastric diversion was only moderately hypertrophied, probably because of impaired nutritional absorption. In all groups, the top of the blind loop was atrophied. Thus, Feny has provided the most recent evidence that the presence of duodenal-pancreatico-biliary secretions are not indispensible for development of compensatory hypertrophic changes in the small bowel.

Neuro-Vasculature Factors: Neuro-vasculature factors are considered in the Innervation Hypothesis of intestinal

adaptation. Ballinger (7) examined whether neuro-vascular pathways may relate to hypertrophy after partial resection of the gut. He performed autotransplantations of gut in dogs, resulting in organ denervation, interruption of lymphatics, and temporary ischemia, and found that after recovery, the dogs lost weight, had protracted diarrhea, and that the autotransplanted segments demonstrated decreased absorption and loss of mucosa and villi. Recovery of intestinal morphological and functional capacity to normal was not complete until a period of 6 months. In another group of dogs, denervation, without subjection to ischemia or lymphatic interruption, resulted in similar results; he therefore concluded that the nervous system must play a major role in structual adaptation.

Delaney (30) found that vagotomy alone was not associated with perfusion rates less than control levels, and concluded that physiologic changes which follow vagotomy should not be attributed to gastrointestinal ischemia. Silen (31) also performed vagotomies in dogs, and found an associated increase in the fraction of epithelial cells in the phase of DNA synthesis in the duodenum and jejunum 6 weeks post-operatively. In reference to Ballinger's results, it was suggested that rapid degeneration of cells might stimulate proliferation of progenitor cells, but that if demand exceeded replacement, there would still be net stunting of the villi. Silen also noted that the number of cells in the crypt was

less than controls, but that the rate of proliferation of epithelial cells was increased and that the total time of replacement of cells was decreased by vagotomy.

Touloukian (8) examined the question of contribution of ischemia to adaptation. He performed blood flow studies and demonstrated the mucosal blood flow was increased within the ileal remnant after enterectomy after just 2 days post-operatively, preceeding evidence of subsequently developed compensatory hypertrophy. In the jejunal proximal remnant, there was no evidence of increased blood flow or subsequent development of hypertrophy. Thus, the increased blood flow to the ileal remnant was selective and correlated with the subsequent development of compensatory hypertrophy. In 1972, Touloukian (32) discovered that massive resection partially denervates the ileal remnant of its adrenergic postganglionic fibers, resulting in increased blood flow and decreased catecholamine activity by 50%. This adrenergic response was not found in the jejunum. Thus, he postulated that increased blood flow may stimulate cellular growth by increasing circulation of a humoral growth factor, or simply by increased local cellular metabolism and cellular growth rates.

Tilson (33) produced ischemia by clamping intestinal segments and found that in both jejunum and ileum villus hypertrophy developed after 7 days; he suggested that ischemia resulted in the release of a systemic "injury

factor" that initiated the adaptation response.

Nygaard (34) also noted that in his experiments the sham-operated controls developed hypertrophy restricted to 2-3 cm's from the site of anastomosis, but that the rest of the intestine appeared normal. He concluded that this hypertrophy could be explained by localized tissue ischemia and poor local circulation at the site of anastomosis resulting in diminished effects of a circulatory inhibitor substance, with consequent unopposed hypertrophy taking place.

Tissue Mass Hypothesis: The tissue mass hypothesis inspired by Weiss (9) has been summarized in the Introduction section of this report, and also considered the existence of a naturally secreted inhibitor substance that could control growth after resections. However, Tilson (35) tested this theory by comparing 75% small bowel resections with 75% bypass of distal bowel, and found 10% and 39% increases, respectively, in jejunal villus height. Thus villus hypertrophy was induced in the bypassed segment without loss of tissue mass, suggesting that the mechanism initiating compensatory growth is not due to loss of an inhibitory substance elaborated by the resected segment.

Functional Demand Hypothesis: Though the focus of modern research of intestinal adaptation was centered upon the intraluminal factors influencing adaptation, it became clear soon enough that other systemic factors must play

a significant role. In 1969, Wright (36) reported the demonstration of increased water resorption, villus length, and villus cell count in the ileum of human ileostomy patients. Wright (37) also demonstrated the same evidence of morphological and functional adaptation in the rat after totel colectomy. Willmore and Dudnick (38) found evidence of hypertrophied intestinal villi in gut remnants of beagle puppies maintained on intravenous hyperalimentation without any oral feedings.

Thus, Tilson and Wright (10) in 1970 designed an experiment that resulted in the development of the functional demand hypothesis. To test the significance of intraluminal factors, they first performed 50% proximal bowel resections accompanied with distal ileal bypass in the rat, and found that both the functioning remnant hypertrophied (villus length increased 61%, mean cell count increased 73%, and migration rates increased 45%) as well as the bypassed remnant (villus length increased 39%, mean cell count increased 54%, and migration rates increased 45%). In a second experiment, distal ileal bypass was followed by colon bypass, and both the bypassed and functional ileum resulted in hypertrophic increase in villus length and cell count. These results suggested a systemic stimulus accounting for the adaptation response seen in the bypassed ileal remnant. The fact that the magnitude of hypertrophy was more pronounced in the functiona ileum than in the bypassed remnant supported

the hypothesis that intraluminal contents had some role in the production of growth, but that intraluminal nutrition was not the exclusive stimulus. It was concluded that loss of functional gut resulted in a demand of the organism for increased absorption of salt, water, and other nutrients, and that this demand was communicated via some unknown hormone back to the gut resulting in compensatory adaptation.

Additional evidence for a hormonal implication behind adaptation was demonstrated by Tilson and Wight (39) by observing that villus hypertrophy was found in parabiotic fed rats following 75% small bowel resections in other rats. In 1972, Tilson (40) reported a clinical case of an infant who had had a laparotomy for proximal intestinal atresia and found to have evidence of villus hypertrophy. Finally, Tilson (35) performed 50% small bowel resections in rats in conjunction with a short (2-3 mm) side to side ileo-ileostomy, compared to a similar resection with a long (20 mm) side to side ileo-ileostomy in order to compare the response in the greater shunt, or bypassed segment of bowel. He found an equal increase in villus height (27%), but greater cell count in the second group with "high functional demand" (greater bypass) and "low intraluminal nutrition" over the first group with the short anastomosis and relatively greater "intraluminal nutrition" and lesser "functional demand".

Further research was continued over controversy of whether both factors of intraluminal effects and

functional demand contributed to intestinal adaptation. While Feldman (41) compared the ileum of jejunectomized dogs fed orally with those fed intravenously and found a significant increase in villus height and glucose absorption in the former, but a decrease in villus height and glucose absorption in the latter, Levine (42) repeated the same experiments, but this time gave the same elemental diet intravenously and orally to the two groups of dogs, and found that the ileum hypertrophied in both groups, although oral feeding induced a greater degree of hypertrophy than did intravenous feeding. Feldman and Dowling (43) came back in 1976 and reported again that 50% proximal small bowel resections in dogs fed orally resulted in villus hypertrophy and increased glucose absorption, alpha-glucosidase, and catalase activity; but that in the same surgically prepared dogs fed intravenously, there was no evidence of functional adaptation and an actual decrease in villus height. They concluded that their results provided "direct evidence that luminal nutrition is essential for development of intestinal adaptation after resection, and that luminal contents are necessary to maintain the structural and functional integrity of normal small bowel".

In 1976, Dworkin et. al. (13) designed an excellent experiment that demonstrated that while intraluminal nutrition presumably maintains small intestinal mass by direct contact with epithelial cells, hormonal or

neuro-vascular factors elicited by feeding may play an important indirect role. They observed the adaptation response of bypassed segments of bowel vs. bowel left in continuity with both groups fed either intravenously or by intragastric infusion. They found that gut weight, mucosal weight, DNA content, and protein content of both gut in continuity and bypassed gut increased significantly, but that intragastrically infused rats in both groups adapted greater than those fed intravenously. Thus, direct contact of intraluminal nutrients influenced gut mass (intragastric-infused rats greater than intravenously fed rats), but also intraluminal nutrients stimulated indirectly hormonal and/or neurovascular factors systemically to increase gut mass (intragastric infused rats had greater mass in bypassed gut than did intravenously fed rats in bypassed gut).

Recently, evidence of a hormonal factor involved in intestinal adaptation has been accumulated by observing the effects of colon resections "downstream" from the site of adaptation in the small bowel. Masesa (44) reported that colectomy in rats resulted in an increase of 33% in small intestinal weight, more pronounced in the upper and lower thirds than in the middle. Villus height was increased 10-12%. However, he attributed this adaptation to a 30-40% increase noted in food intake in colectomized rats, and attributed part of the adaptation response to the consequence of increased intraluminal

nutrition. However, he also demonstrated other factors behind the adaptation response: by pair feeding colectomized rats with controls, after 3 weeks the colectomized rats lost 4% of body weight but their small intestinal weights still increased 17% greater than controls.

In January of 1977, Masesa (45) extended his experiments to examine the effects of hemicolectomies. He found that 3 months after right hemicolectomy in the rat, the left colon remnant weight was greater than controls, but that left hemicolectomy did not produce a similar adaptive change in the remaining right colon remnant. Furthermore, while left colectomy caused no weight changes in the small intestine, right colectomy resulted in a 23% weight increase in the small intestine.

In comparison, our experiment of left colectomy also did not result in significant evidence of hypertrophy in the right colon remnant, though we were able to demonstrate a significantly functional increase in salt and water absorption in the right colon remnant, correlated with functional increases in transmural PD and Na-K-ATPase activity.

Buchholtz (46) found that colectomy in the rat stimulated RNA and DNA synthesis within two weeks throughout the small intestine as well as increaed morphological changes of increased villus height, rates of cell migration, and deeper crypts.

Conversely, Tilson (47) has recently found evidence of early proliferative activity in the left colon of rats after 10 days from 50% resections of distal jejunum and proximal ileum, manifested by increases in crypt height and other proliferative indices. He thus suggested that the whole colon is stimulated as part of the adaptive response to partial enterectomy. Nundy (48) has also recently reported the onset of cell proliferation in the colon following ileal resection. However, McDermott (49) performed autoradiographic studies in rats after 40% intestinal resections and found no change in the mean total number of cells, number of labeled cells per crypt cell column, and sizes of proliferative and mature cell zones over controls.

These latest studies, in combination with our own experimental results, indicate that research on colonic adaptation may not only contribute to portraying the complete picture behind adaptation in terms of its hormonal components, but also shed light towards the progress of understanding small bowel adaptation. Before concluding, it will be helpful to review the latest literature on the physiological, pharmacology, and biochemistry of colonic transport functions as they contribute to the specific mechanisms of functional adaptation. In this manner, the parameters of functional adaptation measured in our experiment (salt and water absorption, transmural PD, and Na-K-ATPase actuvutt), may be placed in perspective.

C. Physiological, Pharmacological, and Biochemical Mechanisms of Functional Adaptation

Absorption and Secretory Function of the Colon:

There have been numerous reviews of colonic absorptive functions (50-59), and though much is known of the colon's capacity to conserve water and salts, how this is specifically controlled, especially in adaptation, is uncertain. Cummings (60) reports that about 1500 ml of fluid enter the colon each day from the ileum. Of this, the normal colon absorbs 1,350 ml of water, 200 mequiv. Cl^-, and 60 mequiv. HCO_3^- each day. The K^+ absorption and secretion is about balanced, depending on the K^+ balance in the organism.

Na is transported actively by the colon and is responsible largely for the electrical gradient across the mucosa. Colonic mucosa has been shown to absorb Na from luminal concentrations as low as 15 mequiv/liter and against a transmural PD of 40 mV. Unlike the jejunum, colonic absorption of Na is not stimulated by glucose, HCO_3^-, or amino acids. Transport of water in colon is passive and occurs in response to solute transport; as Na is the main cation, it closely parallels Na transport. K^+ transport is mainly passive along its electrical-chemical gradient, and the Cl^--HCO_3^- exchange system operates in the colon similarly as in the ileum.

Schedl (61) notes that basic to the considerations of Na and water transport are the physiochemical properties

of cell membranes. Epithelial cells are polar in the sense that the rate of active Na transport is greater at the serosal than the luminal pole; this difference permits the net transport of Na across the epithelial cell.

Wright et. al. (62) have shown that colonic function is altered in states of obstruction: they found that absorption of a test solution increased at moderate elevations of pressure, but then fell below normal at pressures 3-4 times normal. Burg (63) worked on the ascending colon and found that increasing pressure from control values of 4-30 mm's of saline resulted in rapid reversal of net absorption to net secretion. They suggested that an increased intraluminal pressure is transmitted across the mucosa, causing an increase in mucosal interstial pressure with a diffusion of isotonic saline into the lumen in response to this gradient.

Devroede (64) examined regional differences in colonic function and reported that Na flux inward was most rapid in the cecum and decreased progressively towards the rectum. However, when it comes to adaptation, Wright (unpublished data) found that after right and left hemicolectomies in rats, both the remaining right hemicolons and left hemicolons had increased transport of salt and water to a rate equal to the entire colon in intact shams; however, no significant difference in net absorption was found between right and left adapted hemicolons. In contrast, Gazet (65) in a clinical

review of human colonic surgery reported that patients with right hemicolectomies were six times more likely to have significant diarrhea subsequently than patients who had had left hemicolectomies.

Our experimental results indicated that left colectomy in the rat resulted in a functional 103% adaptive increase in water absorption in the remaining right colon; however, this response was abolished by salt-loading the rats. As we shall see, salt loading may have interfered with the initiation of a hormonal factor stimulating adaptive increase in Na-K-ATPase activity.

Transmural Potential Difference: Edmonds has done considerable work on the use of transmucosal potential difference (PD). In 1970, he reported that a normal human colon PD of 25 mV increased to 60 mV after injection of aldosterone. Urinary Na and the Na/K ratioes fell, as well as the Na in stool contents. The decreased Cl^- found in stool was consistent with passive distribution of Cl^- along the electrochemical gradient, but the increased K secretion was greater than could have been accounted for by passive diffusion alone, suggesting a potential mechanism for active K secretion. Edmonds reported that the colonic PD was largely generated by mucosal cells in the course of active transport of Na across the mucosa from lumen to blood, and was raised considerably when Na transport was stimulated by either

Na depletion or injection of aldosterone. Thus he concluded that measurement of colonic PD could become a useful clinical assessment of the functional state of the epithelium.

In 1975, Edmonds (67) recorded that the PD was usually less than 10 mV in the jejunum and ileum of man, but was increased to 30-40 mV in the colon, with little variation along the colon. He discussed that two mechanisms contribute to the transmural PD. When there is a difference of composition between the solutions bathing the two sides of an epithelium, diffusion potentials result from the different mobility of various ions moving through the tissues. Secondly, transfer potentials are associated with active transport of charged particles from one side of the epithelium to the other, and is coupled to the metabolic processes so that transfer PD depends upon the integrity of cellular mechanisms. Thus, when colonic epithelium is examined in vitro, it can be shown that anoxia, metabolic inhibitors (eg. dinitrophenol), and cardiac glycosides (eg. ouabain), will reduce or abolish the PD and eliminate active transport (68).

Archampong (69) studied colonic PD's and concluded that because Na is the major ion actively transported against the electrochemical gradient, and net Na flux is closely correlated with the short circuit current in human colon studies in vitro, that it is likely that active Na absorption is the major factor in the transfer PD of colonic mucosa.

It has been demonstrated that transmural PD can be influenced by hormones and drugs: Edmonds (70) reported that aldosterone infusion in the rat nearly doubled the PD within a few hours, and that the change in PD was greatest in the distal colon and rectum. Thompson and Edmonds (71) reported that when the epithelium was exposed to stimuli such as aldosterone or Na depletion which increased the Na absorptive rate, that there was no significant increase in Na-K-ATPase activity. They concluded that the mechanism of aldosterone was not through an effect on Na-K-ATPase, but rather possibly in controlling the Na access to the Na pump, or to the provision of energy to the pump. However, they did report that hypothyroidism resulted in decreased Na-K-ATPase activity, and therefore that thyroid hormones have a significant role in influencing electrolyte transport across epithelia and cell membranes.

In contrast, our experimental results demonstrated that left colectomy in the rat resulted in a 144% increase in Na-K-ATPase activity over controls, presumable by the initiation of salt-depletion evoked stimuli, because salt-loading left colectomized rats abolished any significant adaptive change in Na-K-ATPase activity. Correlated with the normal adaptive increase in Na-K-ATPase activity, we found that left colectomy in the rat also resulted in an increase of 29% of the distal right colon remnant PD and an increase of 18% of the proximal right colon remnant PD over controls, and that both these increases

were abolished by salt-loading left colectomized rats.

Isaacs (19) examined PD changes in human ileostomy patients, and reported that PD increased as a function of time after colectomy and also increased as measurements were made closer to the tip of the ileostomy. They also found that spironolactone decreased the transmural PD. They reported a decrease in the salivary Na/K ratio in ileostomy patients as an index of increased aldosterone activity, but were unable to demonstrate a quantitative increase in aldosterone levels. They concluded that the ileostomy patients suffered mild salt depletion that resulted in increased aldosterone sensitivity, followed by increased absorption of Na correlated wtih increased PD's in the ileum.

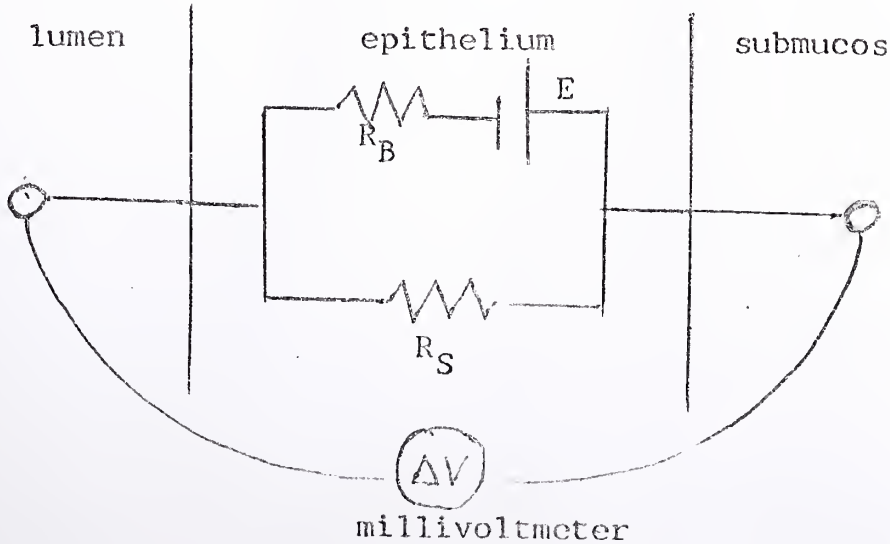
Edmonds (67) designed a model of an equivalent circuit of transmural PD as follows:

E = EMF generated by active transport.

ΔV = transmural PD.

R_B = internal resistance.

R_S = shunt resistance (passive movement of ions through epithelium).



The model demonstrates that a change in measured PD may be due either to an alteration in the activity of the Na pump (E) or to a change in the shunt resistance (R_S).

For example, if a tissue becomes "leaky", the freer movement of ions leads to reduction of R_S and hence a reduction in ΔV, although the E may be unchanged. Thus the functional state of the epithelium influences the PD. Edmonds reported that in acute proctocolitis, the PD decreased to practically zero, and stimulation with mineralocorticoids produced no effect upon restoring the PD. Associated with the decreased PD was considerable impairment of Na absorption and increased permeability; thus, he concluded, it seems likely that in colitis the decreased PD results both from cellular injury and damage to the Na pump mechanism (reduction in E), and to increased epithelial permeability (decrease in R_S).

Salt-Depletion, Mineralocorticoids Effects, and Na-K-ATPase Activity: The specific controlling mechanisms of intestinal adaptation have been investigated as a result of observed variations in absorptive functions of the adapted gut. Kramer (72) reported that in ileostomy patients who were salt depleted, the small intestine was found to conserve Na and secrete K, and that the total mass of the ileostomy output was proportional to the amount of Na and K in the effluent. Clarke (73) reported also a decrease in the Na/K ratio in an ileostomy patient which was restored

to normal when salt was administered to the patient. Clarke (74) also found that spironolactone increased the excretion of Na from ileostomy patients, resulting in decreased Na/K ratioes, but that again when these patients were given salt the Na/K ratioes returned to normal. Goulstrone (75) studied the effects of mineralocorticoids on ileostomy patients and found that administration of 9-alpha fluorohydrocortisone decreased the Na/K ratio in ileostomy effluent by 30%. Levitan (76) also reported increased Na absorption and K secretion in human ileostomy patients after administration of aldosterone. Stein (77) had reported in 1941 that adrenalectomy depresses Na and water absorption by the gut, but that this depression was reversed by administration of DOCA. Shields (78) also implicated the possible role of the adrenal cortex in intestinal adaptation by noting the association between salt depletion, increased Na absorption and K secretion, and Clark (79) reported ileal conservation in dogs subjected to Na depletion.

Crocker (80) prepared everted sacs of rat jejunum and found that rats who had been kept on low salt diets demonstrated increased absorption of salt and water in their in vitro preparations, and similarly, that increased in vitro absorption was found in salt-loaded rats that had been given aldosterone prior to sacrifice. Spat (81) also reported increased Na absorption and K secretion in rat intestine after aldosterone administration, and that these effects were more pronounced in the ileum than the colon.

The association of intestinal adaptive changes in absorptive functions with the influence of Na balance and mineralocorticoids led to investigation of hormonal influences upon enzymatic transport mechanisms. Morphological control mechanisms resulting in compensatory hypertrophy of the small intestine were reviewed by Altmann (82). He noted that mucosal cells are produced from mitosis in the crypts and then pushed outwardly to be incorporated into the villi, and subsequently sloughed off at the apical region. Quasar (82) had postulated a "critical zone" between the lower 2/3 and upper 1/3 of the crypt since, when passing through that zone cells would no longer take up thymidine. Thus, in compensatory hypertrophy, it was suggested that production of cells in the crypt was greater than destruction at the apical region, resulting in increased villus length. Altmann concluded that sometime prior to the mitotic block at the critical zone most of the RNA is already synthesized. This RNA would initially be responsible for the synthesis of proteins for the microvilli and apical zones as well as absorptive enzymes, and later synthesize the constituents necessary for cellular migration; when the RNA pool eventually became consumed, the cells would slough off. Thus, control of hypertrophy was found to be indexed by the size of the critical zone and the amount of RNA present in the cells at the "committed point" level of the cellular migration.

Porter (84) experimented with frog bladders and demonstrated that administered isotopic labelled aldosterone was found in the epithelial nuclei; furthermore, the subsequently increased Na transport could be blocked by actinomycin D and puromycin. They were able to document an immediate increase in RNA concentrations following aldosterone administration, and concluded that aldosterone acted through a mechanism of stimulating RNA synthesis in the nucleus. Castles (85) demonstrated similar results in the kidney suggesting that mineralocorticoids may induce the synthesis of RNA programmed to increase the synthesis of protein enzymes.

Schultz (86) suggested that the adaptation response was mediated through an increase in Na-K-ATPase because ouabain, a well known inhibitor of Na-K-ATPase, was found to reduce the Na transport in the gut. Tilson and Wright (87), (88), found increased Na-K-ATPase activity in the ileal remnants of rats after partial resections as well as in transposed ileal segments into the proximal jejunum. However, Weser (89) was unable to demonstrate significant changes in the disaccharidase activity of lactose, sucrose, or maltose after small bowel resection. Thus the variable regional sensitivity of intestinal enzymatic adaptation became apparent.

In 1969, Quigley (90) was able to demonstrate that the lateral plasma membrane of intestinal epithelial cells had the greatest Na-K-ATPase activity, and Tilson and Wright (91) relied upon electron microscopy to demonstrate

a marked increase in the size of the lateral membrane of ileal enterocytes following massive small bowel resection, and concluded that ileal adaptation included an increase in the level of transport enzymes per cell, either as a result of increases in cellular enzyme concentrations at the plasma membrane, or as a result of increased plasma membrane itself.

Fenoglio (92) has employed scanning electron microscopy to depict features of hyperplastic colonic polyps, and found increased cellular diameters and larger microvilli than in normal colonic mucosa. He also identified that hyperplastic polyps are covered with overdeveloped absorptive cells, and that the normal territorialization of cells remained present but distorted.

Tilson and Wright (93) continued in 1971 to accumulate data on the hormonal inducement of morphological and functional adaptation. After administering DOCA to rats for 7 days they were able to simulate compensatory hypertrophy. They found increased villus length and cell migration rates, a 28% increase in Na-K-ATPase activity, and an increased absorptive capacity for salt and water in the rat ileum.

In 1975, Schmidt (94) presented evidence that Na-K-ATPase in the proximal and distal convolutions and thick ascending limb of the loop of Henle in rats was a possible target for the action of aldosterone. After adrenalectomy, the Na-K-ATPase activity in these locations was decreased, but treatment with aldosterone returned the level of activity to normal within 1 hour. This rapid activation

of Na-K-ATPase induced by hormone was completely blocked by actinomycin D and cycloheximide, thereby implying that the aldosterone effect on Na-K-ATPase requires an intact protein synthetic process.

Oya and Weser (95) reported that after small bowel resection in the rat, there was a reciprocal decrease in adenyl cyclase associated with the adaptive increase of Na-K-ATPase activity. Corriveau (96) had presented evidence of cytochemical localization of adenyl cyclase in the basement membrane, in the junctional area between two epithelial cells, and on the brush border of all epithelial cells in the descending colon of the rat, but only on the brush border of cells in the ascending colon.

Yau (97, 98), using a muscle-stripped everted open sac preparation of rat colonic segments, was able to demonstrate that administration of dibutyryl cAMP, as well as theophylline, resulted in a reduction of net water flux by 38% and 23% respectively. He also demonstrated that the net water and solute fluxes were similar in both ascending and descending segments of colon, but that specific ionic components of solute fluxes were different: the descending colon absorbed less Na, secreted more HCO_3, and exclusively absorbed K; while the ascending colon absorbed more Na and no potassium. Therefore, he concluded that the ascending colon and descending colon in the rat displayed quantitative differences in Na absorption and HCO_3 secretion, but qualitative differences in K transport.

The role of aldosterone has also been studied for its effects on epithelial adaptation to K balance (99), (100), (101). These investigators found that chronic K loading (7 days) induced an increase in Na-K-ATPase activity in rat colonic mucosa. After chronic K loading, the K secretion increased, the transmucosal PD increased, and the Na-K-ATPase increased; however, colonic movement of Na, Cl, and water remained the same as controls. They concluded that the increase in Na-K-ATPase activity was associated with the increased PD and K secretion, and was important for the control of transepithelial movement of K. Silva (102) reported that adrenalectomy abolished the effect of stimulated Na-K-ATPase activity in K-loaded rats. Thus, the increased secretion of aldosterone normally evoked by K loading appears to mediate at least in part for the increased Na-K-ATPase activity resulting from K loading.

The influence of aldosterone upon intestinal adaptation raised the question of possible direct involvement of angiotension. Dolman (103) noted that in vivo administered angiotensin had no significant action on PD or on ionic fluxes of proximal or distal colon in the rat. However, transmucosal PD was increased with the administration of aldosterone; also the effect of Na depletion in stimulating Na absorption and K secretion was completely abolished by adrenalectomy, but unaffected by nephrectomy. Dolman also reported regional differences in the aldosterone

response in the rat colon: while aldosterone and Na depletion both stimulated Na absorption in both proximal and distal colon, significant increase of K secretion was demonstrable only in the distal colon. In the proximal colon, the increased Na absorption appeared to be accompanied by increased Cl absorption while in the distal colon it was principally the Na-K exchange that was increased. Adrenalectomy decreased K secretion in both proximal and distal colon, but Na absorption was only significantly decreased in proximal colon. Thus, the investigators concluded that there was no evidence that angiotension in vivo had a role as an important salt retaining hormone by direct epithelial action; aldosterone had a considerable effect which was independent of angiotension, and which differed in proximal and distal colon in regard to the relative effects on Cl absorption and K secretion.

Levens (104) performed in vitro experiments and demonstrated that angiotensin directly stimulated fluid transport in the colon, but in the absence of any change in transmural PD or resistance. Thus, he concluded that angiotensin exerted its action via an electroneutral process in some way contrasted with the mechanism of aldosterone, which stimulated eletrogenic Na transport.

Most recently, Munday (105) has found that angiotensin had no effect of either the cAMP contents of adenyl cyclase activities in rat colon nucosa or kidney cortex.

The investigation of other possible hormonal contituents of the intestinal adaptation response has been extensive.

Charney (106) reported that while DOCA increased Na and water absorption, K secretion, transmural PD, and Na-K-ATPase activity only in the rat colon, glucocorticoids (methyprednisolone acetate) were found to increase these functional parameters of adaptation in the jejunum and ileum as well as in the colon. Kisloff (107), seeking the mechanism of diarrhea in 30% of patients with thyrocalcitonin-secreting medullary carcinoma of the thyroid, found that intravenously infused thyrocalcitonin resulted in net secretion of water and electrolytes in both jejunum and ileum of the rabbit.

Effects of prostaglandins have been studied (108-110) and found to result in net water and electrolyte secretion in the ileum, but to have no significant effect upon the colon. The relative order of potency causing secretion was PGE_1 followed by PGE_2 and lastly PGF_{2a}.

Effects of bile acids on colonic secretion have also been studied. Mekhjian (111) reported that predominantly dihydroxy bile acids, whether free or conjugated, inhibited absorption and induced reversible secretion of Na and water in the colon. This effect was related to the intraluminal concentration rather than to the amount of bile acids absorbed. The proposed mechanism was that bile acid anions are surface active molecules that could impair active Na transport by altering enzyme configuration, by interfering competitively with the binding of phosphate, or by altering the lipid-protein interactions in cellular membranes.

Ammon (112) demonstrated that C-18 fatty acids inhibit water absorption in both colon and proximal jejunum and can cause water secretion in small and large intestine, accounting for the association of steatorrhea and diarrhea. Conley (113) reported that cAMP may be the mediator of bile acid induced colonic secretion because adenyl cyclase was increased proportionately with increasing concentrations of deoxycholic acid (DCA); they also reported that cAMP infusions mimicked the physiological effects of DCA. Meanwhile, Coyne (114) reported that the DCA stimulation of colonic adenyl cyclase was inhibited by the B-adrenergic blocking agent, propranolol. Thus it was concluded that bile acids induced colonic secretion by their effects on the synthesis of cAMP.

Finally, Field (115) reported that epinephrine or norepinephrine, by alpha adrenergic actions, blocked the effects of PGE on increasing cAMP levels and stimulating salt and water secretion in the rabbit ileal mucosa.

SUMMARY

In conclusion, our experimental results indicated that left colectomy in the rat did not result in detectable evidence of morphological adaptation, but did manifest evidence of functional adaptation in the remaining right colon in terms of significantly increased salt and water absorption, transmucosal PD, and Na-K-ATPase activity; furthermore, these adaptive changes were abolished by salt-loading left colectomized rats.

These results suggest that the intestinal adaptation in the right colon resulting from the effects of "downstream" left colectomy is not dependent upon intraluminal contents, but rather is mediated by some systemic hormonal factor that is elicited presumably by a state of salt depletion since salt loading abolishes the adaptive response. Review of the literature on intestinal adaptation has demonstrated repeatedly that conditions of salt depletion lead to increased aldosterone secretion and stimulation of membrane receptors which decrease adenyl cyclase, decrease cAMP, and ultimately result in increased Na-K-ATPase activity correlated with the increased transmucosal PD and salt and water absorptive capacities demonstrated at the site of the adapted intestine.

Adaptation response has been found to differ among regional areas of the bowel, and specific control mechanisms have not been developed to explain exactly how adaptation occurs along the regional distributions of the small and

large bowel. Further research will be required on both large and small bowel preparations in order to elucidate the intricacies of these specific control mechanisms of adaptation; as a result of this understanding, medical science may employ the knowledge to maximize the adaptational response in order to clinically improve the post-operatively consequences of small and large bowel surgery as well as to manipulate the sequelae of intestinal disease.

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